



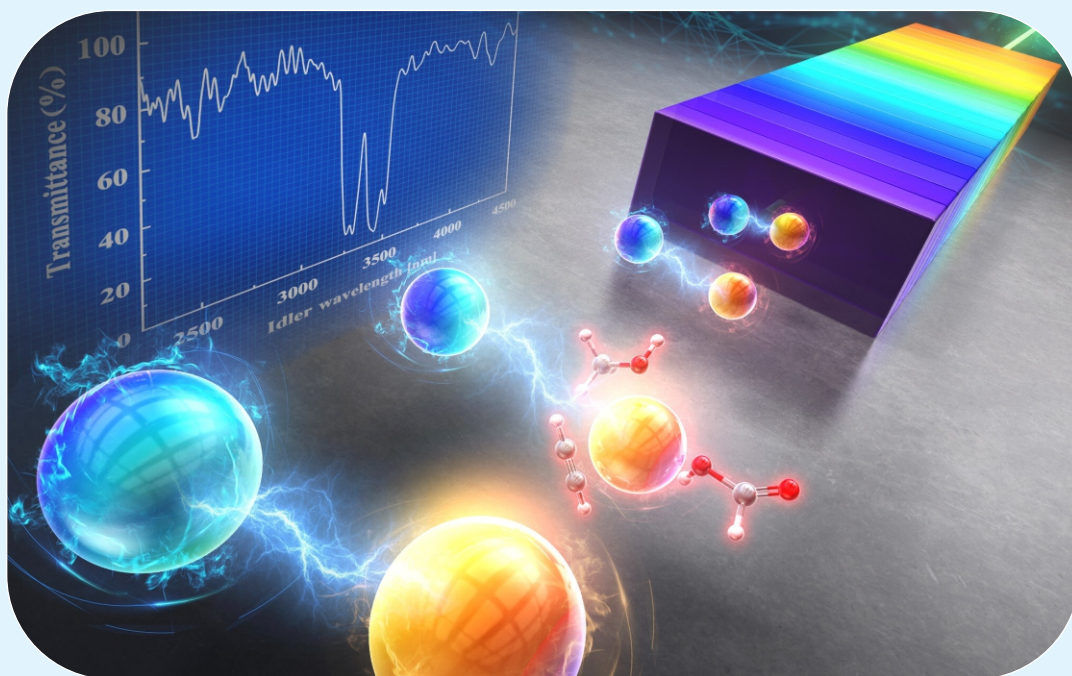
**MATS**  
UNIVERSITY

NAAC  
GRADE **A<sup>+</sup>**  
ACCREDITED UNIVERSITY

# MATS CENTRE FOR OPEN & DISTANCE EDUCATION

## Spectroscopy I

Master of Science  
Semester - 1



**SELF LEARNING MATERIAL**



**CC 04**

**SPECTROSCOPY I**  
**MATS University**

**Spectroscopy**  
**CODE: ODL/MSS/MSCH/104**

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## **MODULE -INTRODUCTION**

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Course has Five module. Under this theme we have covered the following topics:

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	Unit 14	Chemical Shift Scale
	Unit 15	Advanced NMR Techniques

These themes of the Book discuss about Spectroscopy, which is a technique used to analyze the interaction between matter and electromagnetic radiation. It is primarily used in physical and analytical chemistry to detect, identify, and quantify information about atoms and molecules, as each has a unique spectrum. This book is designed to help you think about the topic of the particular Unit. We suggest you do all the activities in the Units, even those which you find relatively easy. This will reinforce your earlier learning.



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**Module 1**

**UNIFYING PRINCIPLES AND MICROWAVE SPECTROSCOPY**

**Unit -1 Introduction to Spectroscopy**

The spectrum of light is nothing bridging to the revolutions in the historical science, becoming the most sophisticated scientific method of enlightening a different world of matter and energy by looking deep into the fundamentals of chemical and physical nature and their interaction with space or electromagnetic radiations. Fundamentally, it is an analytical methodology that examines the interaction of various types of materials when exposed to electromagnetic radiation, enabling scientists to gain unparalleled access to the structural, compositional, and energetic properties of atoms, molecules, and complex systems that span several scientific fields. The early beginnings of spectroscopy as an area of research stem from the 19th century explorations of the interaction between light and matter and has included many iconic contributions from pioneering researchers aiming to decode the astrophysical landscape of electromagnetic interactions. The stages through which light passes first among mystics and the faithful, later with the experiments, and eventually the speck of the atom, the bearded face, the prism, and then as it disperses, and then nothing, just your efforts are still, at quantum level, just the dissimilarities in the spirit that are used to represent the different types of psychedelic crystals in the Bowie and Williams of your choosing. In the years that followed, scientists such as Joseph von Fraunhofer built on those discoveries, carefully studying the dark lines in solar spectra and demonstrating that different materials have unique spectral signatures that can be used to identify and characterize their essential properties. Spectroscopy is a scientific field that is defined by the interaction between electromagnetic radiation and matter. This definition is more than just a measurement technique; it is a powerful analytical framework that enables researchers to examine the quantum-mechanical traits of everything from individual subatomic particles to entire astronomical objects.

They provide scientists the ability to obtain rich information on a material's composition, structure, energy states, and dynamics by measuring electromagnetic radiation absorption, emission, and scattering as a function of wavelength. Why so, Well, Spectroscopy is based on Quantum Mechanical Model, which states



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that energy exchange takes place between matter and radiation quantized in separate packets. Electromagnetic radiation can be absorbed by a material in which case the energy is transferred to the material emitted from a material (the material done it to transfer energy to the radiation) or scattered (the radiation is redirected by the material with little or no energy lost to the material). These interactions yield distinct spectral fingerprints, providing a powerful window into the quantum mechanical activity underlying the system under investigation. Electromagnetic radiation, in many respects, is the foundation of spectroscopic analysis, acting as both the probing medium and source of information about material properties. This extraordinary type of energy moves across the cosmos in the form of oscillating electric and magnetic fields, propagating at the speed of light and described by their own unique properties of wavelength, frequency and energy. The wide range of types of physical processes that can be monitored spectroscopically can be achieved only through the vast diversity of radiation types offered by the electromagnetic spectrum. Electromagnetic radiation is fundamentally quantum mechanical in nature, best understood through the concept of wave-particle duality. This groundbreaking realization indicates that electromagnetic radiation can demonstrate dual natures, possessing wave-like and particle-like properties, contingent upon the experimental conditions. The double-slit experiment Daniel P. Hines, “Interference and diffraction” image–source peratt.com Electromagnetic radiation carries with it energy proportional to its frequency and inversely proportional to its wavelength as elegantly expressed by the Planck-Einstein equation,  $E = h\nu$ , where ‘E’ is energy, ‘h’ is Planck’s constant and ‘ $\nu$ ’ frequency.

#### **Electromagnetic Radiation and Its Properties**

The diverse regions of the electromagnetic spectrum offer complementary spectroscopic investigation opportunities with varying advantages and intellectual input into material property. The longest wavelength radio waves of the lowest energy are used in nuclear magnetic resonance spectroscopy. Rotational spectroscopy is made possible by microwave radiation, while molecular vibrations can be studied by infrared radiation. Visible light spectroscopy enables the analysis of color, ultraviolet radiation probes electronic transition, while high-energy X-rays and gamma rays shed light on atomic and nuclear processes (Anderson et



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al., 2017). Although spectroscopic techniques can achieve such an extraordinary level of detail and precision, this comes as no surprise from the quantum mechanical nature of electromagnetic radiation. As radiation strikes atoms or molecules, electrons can move to transitions between energy levels, gaining or losing energy in fixed quantities. The resulting absorption or emission spectra of these quantized energy transitions lead to characteristic lines or bands of light at particular wavelengths, acting as fingerprints of a material's electronic structure, chemical composition, and energy states. This fundamental principle enables researchers to extract more information about matter at the atomic and molecular scales than ever before. Spectroscopy in the past originated from the collaborative studies of physicists, chemists, and astronomers who realized the importance of studying the interaction between radiation and matter. Over the years, advanced instrumentation has played a major role in improving the accuracy and sensitivity of spectroscopic measurements, resulting in devices such as prisms, diffraction gratings and modern spectrometers. Such advancements have evolved spectroscopy from a qualitative observational technique into a quantitative analytical approach with remarkable molecular and even atomic resolution. The wavelength of electromagnetic radiation is a critical parameter in spectroscopic analysis as it determines what type of interaction occurs and what information can be extracted from a single measurement.

Wavelengths vary from the thousands of kilometers long radio waves to the pedometer long gamma rays. This enables scientists to investigate phenomena along multiple scales/energy ranges as each wavelength region allows us view different properties of matter with different insight. Frequency is another central characteristic of electromagnetic radiation that is critical to spectroscopic studies. Frequency is directly related to wavelength via the speed of light and describes how many wave oscillations occur in a second. The higher the frequency, the higher the energy radiation, which allows for more energetic interactions with matter, accesses different quantum mechanical transitions. Spectroscopic observations can be accurately predicted and understood by researchers using the relationship between frequency and energy. Electromagnetic radiation has an energy associated with it, which is a defining factor for how the radiation will interact with matter. Using the Planck-Einstein equation where energy is linear to frequency and inverse to wavelength. This mapping allows





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researchers to predict and comprehend the quantum mechanical transitions facilitated by spectroscopic measurements, providing a theoretical basis for the interpretation of complex spectral data.

Spectroscopic methods can be classified according to the kind of interactions between radiation and matter. It is based on absorption spectroscopy recording the absorption of light by a sample for a set of different wavelengths. Emission spectroscopy is an analytical technique where one studies the radiation, released spontaneously from excited atoms or molecules, to determine their energy polities and composition. Scattering spectroscopy studies radiation deflection by matter to provide insight into molecular dynamics and structural properties. The electromagnetic spectrum depicts a continuous range of types of radiation with different properties and applications in spectroscopy. From radio waves with low energy to gamma rays with higher energy, each domain illuminates unique opportunities to probe material properties. Determined by what specific trait they want to study, researchers can choose wavelength regions, and this helps them carry out spectroscopic analysis in a detailed and relatively complete way. The field of spectroscopic instrumentation has advanced over the last several decades, and includes a suite of optical components, detectors, and computational algorithms that allow for more sophisticated designs and capabilities. Fourier transform spectroscopy and other advanced techniques have led to great improvements in resolution and sensitivity within multiple regions of the radiation spectrum, allowing investigators to obtain increasingly high-fi and accurate measurements of material properties. The fact that spectroscopy is an applied science shows up in the various scientific fields in which it is applied, due to its nature as an interdisciplinary field. Astronomers analyze the composition and movement of cosmic bodies millions of light-years away using spectral techniques. Chemical researchers use these approaches to study molecular architectures and track complicated reactions. Medical practitioners utilize spectral methods for noninvasive imaging and medical diagnostics, while environmental scientists use spectral methods to measure pollutants and monitor changes in environments. Spectroscopy stands to witness ongoing innovation and expansion in the future, with developments in quantum technologies, artificial intelligence, and computational approaches. Newer methods

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such as coherent multidimensional spectroscopy provides unique windows to ultrafast molecular dynamics, while quantum sensing strategies constitute a paradigm shift for measurement precision. I think about these breakthroughs and advances of matter and how in short hand, we have much to learn still.

Spectroscopy, at its heart, is a graduate of our frustrations of the scientific curiosity that can tinker with unseeable brute, magnum opus. Researchers can decode the elaborate mechanisms that shape the behavior of matter by mapping these quantum mechanical phenomena onto observable spectral patterns. To study the fundamental nature of reality, spectroscopic techniques provide a lens from the atomic to the cosmological scale.

**1.1 Interaction of Electromagnetic Radiation with Matter**

Between electromagnetic radiation and matter is a very basic physical phenomenon existing at the heart of physics, chemistry, astronomy, materials science, etc. It is an intricate process involving mechanisms of interaction between electromagnetic waves and atomic and molecular structures that provide deep understanding of the quantum mechanical nature of matter. Such an understanding of these interactions is an important analytical tool for covering the fundamental properties of materials and their electronic configurations, including the states of their energy. At a very basic level, electromagnetic radiation interacts with matter via fairly complex processes that are sensitive to both the properties of the radiation and the material. These interactions follow quantum mechanical laws that describe the exchange of energy between electromagnetic waves and a substance's electrons, atoms, and molecules. The interactions become complex due to the wave-particle duality characterizing electromagnetic radiation and the quantized nature of the available energy levels present in matter, leading to a fascinating interplay between energy and information.

**Absorption, Emission, and Transmission**



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Absorption is one of the most significant and instructive reactions between electromagnetic energy and matter. Absorption is the transfer of energy from electromagnetic waves to material electrons when they shift between different quantum mechanical energy states. This process is very selective and dictated by the electronic arrangement of atoms or molecules, resulting in specific absorption spectra that serve as unique identifiers of material composition. This indicates that each distinct material absorbs radiation at certain wavelengths, corresponding to transitions in the atomic and molecular energy levels inside the material, so offering researchers an effective method to identify and analyze various compounds. This absorption process is a complex interaction dictated by quantum mechanics, wherein photons are absorbed by electrons, elevating them to a higher energy state. This transition occurs when the energy of the photon precisely matches the energy difference between two quantum states of the material. When the absorbed energy aligns with specific energy differentials, electrons can transition from ground states to elevated excited states. These factors encompass the material's electrical configuration, its temperature, and the frequency and intensity of the incident radiation. Emission is the converse of absorption, as it pertains to the transition of excited electrons to lower energy levels, accompanied by the release of electromagnetic radiation. When electrons transition from higher to lower energy levels, they emit photons with energy corresponding to the difference between those states. This phenomena elucidates numerous important scientific and technological applications, including fluorescence, phosphorescence, and other spectroscopic techniques. The emission spectrum of a material is a key attribute that provides insights on its electronic structure, composition, and energy state. Noteworthy among these is thermal emission, in which materials release electromagnetic radiation based on their temperature. Planck's law asserts that all objects above absolute zero emit electromagnetic radiation, with the spectrum and intensity contingent upon the material's temperature. This principle is the foundation for infrared imaging, thermal analysis, and the radiative properties of materials, which span many scientific and technological sectors. Transmission refers to the transmission of electromagnetic radiation through a substance that provides detailed information on the optical and electronic properties of this



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substance. When radiation interacts with matter, it is not all absorbed or reflected, but some of it transmits through and it may be modified by the passage; the intensity, phase, and/or polarization. The thinner the material, the more discerning its transmission characteristics are, which gives researchers more information about its fundamental properties based on how electrons interact with it.

#### **Reflection, Dispersion, and Polarization**

Reflection happens when some electromagnetic radiation interacts and a boundary between two different media and is returned into media. This phenomenon is governed by the optical properties of the materials, such as their refractive indices and surface features. It is thus said that reflection can be specular, when the radiation in response is reflected according to the angles mentioned (according to incidence angle), or diffuse, when the radiation is emitted into various directions. The ability of materials to reflect light has important relevance to optical devices, telecommunications, and the study of the surface of materials. Dispersion is the separation of electromagnetic wave(s) into its component wavelength(s) because they travel through a material at different speeds. This process explains why rainbows are formed and gives a basic mechanism for spectroscopic analysis. The degree to which this radiation separates, varies with the material's optical properties and the wavelength of the radiation, allowing researchers to analyze the composition and electronic structure of materials via accurate measurements of the wavelengths over which the radiation is dispersed. Polarization describes how the oscillating electric and magnetic fields of electromagnetic radiation are oriented. The polarization of radiation can change when it interacts with matter, which allows more information about the electronic and molecular structure of the material. The role of polarization effects in light-matter interactions also underpins a range of applications, from optical communications, through materials characterization, to advanced imaging methods.

#### **Scattering Mechanisms**



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Scattering mechanisms are a heterogeneous and complex variety of interactions wherein EMR is re-routed through the transmission of energy through particles or structural inhomogeneities in the material. There are several scattering mechanisms, and each reveals information about the material properties in its own context. Rayleigh scattering, which is to do with particles far less than the wavelengths of radiation in question, explains things like the blue color of the sky. Raman scattering also produces very specific information about molecular vibrations and rotational states, and Mie scattering is relevant to interactions with particles of comparable radius to the radiation wavelength. This redirection of radiation without change in energy is called elastic scattering and preserves the original wavelength and frequency. Inelastic scattering, on the other hand, means that some energy is exchanged between the radiation and the scattering medium, leading to shifts in wavelengths. For example, this inelastic scattering is especially important, as it gives us detailed information about molecular vibrations, which allows it to be used as an instrumental analytical technique in chemistry and materials science, which we call Raman scattering. The complexity and specificity of the interactions of electromagnetic radiation and matter is a consequence of the quantum mechanical nature of these basic interactions. In solid-state materials, electrons are only permitted to reside within certain energy levels, and transitions between those levels involve well-defined energy exchanges. This discrete behavior gives rise to the absorption and emission spectrum which are the fingerprints of different materials are powerful and advanced analytical tools applicable in many scientific fields. Such interactions between radiation and matter are harnessed in advanced spectroscopic techniques to investigate material properties with remarkable precision. Methods such as absorption spectroscopy, emission spectroscopy, and Raman spectroscopy are powerful tools for characterizing electronic structures, molecular configurations, and energy states. Approaches such as characterization of material interaction with electromagnetic radiation over a broad range of wavelength regimes provide global-level information on the basic properties of materials. Within the scientific quest, there are wide ranging applications of the interaction of electromagnetic radiation with matter that are critical for technology advancement. Examples include; Optical communications;



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medical imaging; materials characterization; astronomical observations; and environmental monitoring all of them depend on sophisticated understanding of radiation-matter interactions.

These interactions offer a meaningful analytical instrument for diagnosis and understanding for the physical world at aggregate scales and higher. With every passing year, technological innovations stretch our opportunities to study and exploit radiation-matter interactions. These complex processes are measured and understood with ever more accurate instrumentation, computational modeling, and quantum mechanical theories. Frontiers in quantum optics and nanophotonics hold the potential to yield even deeper insights into the mechanisms governing photon interactions with matter. This alphabet of interactions ranges a spectrum: from plasma to radio, from spectroscopy to cosmology; from high-energy physics to neutrino detection; from liquid scintillation counters to charge-coupled devices. Researchers investigate the subtle quantum mechanical interactions that determine the physical reality by understanding energy and information exchange between radiation and matter. Whether at the microscopic level, with atomic interactions, or at the macroscopic level with astronomical observations, radiation-matter interactions provide a meaningful way to understand the fundamental nature of the universe. Continued cross discipline work is helping better understand these complex interactions. Theoretical physicists add theoretical framework, chemists design advanced experimental methods, materials scientists build novel instrumentation, and computationists create novel analysis algorithms. Such collaborative platforms will also enable us to distil the studies of radiation-matter interaction, which have traditionally been confined to specific applied science fields, into such powerful and adaptable sets of research techniques that have relevance across a number of scientific disciplines. This trend will continue with the advent of quantum technologies, artificial intelligence, and computational techniques that will leave behind our wildest imaginations — both in terms of understanding and applications. New methods such as ultrafast spectroscopy, quantum sensing, and advanced computational modeling enable us to study how electromagnetic radiation interacts with matter with unprecedented insight into the underlying dynamics. Revolutionary new tools which open the gate to deeper



knowledge about the fundamental properties of matter and its interactions well beyond current models..

### Unit II Fundamental Theoretical Concepts

We provide notions from quantum mechanics and atomic physics, which are based on essential theoretical principles that characterize the behavior of systems at atomic and molecular scales. The fundamental concepts from these Units possess extensive applicability in fields like as spectroscopy, quantum chemistry, and atomic transitions. This section covers the uncertainty principle, natural line width and broadening, transition probabilities and selection procedures, time-dependent perturbation theory, and the Born-Oppenheimer approximation. All of these factors are crucial for comprehending atomic and molecular processes, especially regarding transitions between energy levels, interactions with electromagnetic fields, and the quantum behavior of particles.

#### **Principle of Uncertainty**

The Heisenberg uncertainty principle, often known as the uncertainty relation, is a fundamental tenet of quantum physics. It articulates a fundamental impossibility regarding the precision of specific pairings of physical attributes, namely location and momentum, that can be simultaneously known or measured. The product of the errors in the measurements of location ( $\Delta x$ ) and momentum ( $\Delta p$ ) is theoretically described as being at least a constant value of two:

$$\Delta x \cdot \Delta p \geq \frac{\hbar}{2}$$

where  $\hbar$  represents the reduced Planck constant. First stated by Werner Heisenberg in 1927, this principle follows directly from the wave-particle duality of matter a hallmark of the quantum mechanics. In the classical mechanics, we can know with arbitrary precision the position and momentum of a particle. But the uncertainty principle in quantum mechanics imposes a fundamental limit to the knowledge of these quantities. This uncertainty is not because our measurements are imperfect, but because some is an intrinsic feature

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of quantum systems. The uncertainty relation has far-reaching consequences for atomic and molecular systems. For instance, this means that particles like electrons cannot possess both an exact position and momentum. If one of these quantities is measured more accurately, the other can be known with less accuracy. The fact that we can only know some properties of the particles with a certain precision, means that transitions between them has the behavior we have described, for example, during the emission and absorption of photons the quantum fluctuations affect the energy levels of the atoms or molecules.

### Natural Line Width and Broadening

Atomic and molecular spectroscopy YAG laser parametric accelerator The interaction between an atom or molecule and radiation, characterized by the absorption or emission of radiation, establishes the relationship between the energy of the photon and the energy difference between two quantum states. However, those changes are not distinctly delineated. The intrinsic line width arises from the finite lifetime of the excited state of the atom or molecule. Due to the limited duration of the state's existence, the energy-time uncertainty principle indicates an uncertainty in the state's energy.

Lifetime of an excited state and the natural line width are related through:

$$\Delta E \cdot \Delta t \geq \hbar$$

where  $\Delta E$  is the uncertainty associated with the energy of the state and  $\Delta t$  is the lifetime of the excited state. The wider the spectral line, the shorter the lifetime, and vice versa; the longer the lifetime of the excited state, the narrower will be the spectral line. This effect is commonly known in spectroscopy as the natural broadening of spectral lines and is an inherent trait of the system. But natural broadening is not the only type of broadening that can occur in atomic and molecular spectra. Additional broadening may occur if there are external factors involved, like influences from collisions with other particles or electromagnetic fields. This





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encompasses Doppler broadening, pressure broadening, and broadening due to collisions of the emitting particle in dense media.

### Transition Probability and Selection Rules

In quantum physics, transition probabilities quantify the likelihood of shifting from one state to another as a result of external influences, such as electromagnetic radiation. The absorption or emission of a photon by an atom or molecule corresponds to a transition between two energy states. The transition is feasible if all conditions are met, including the type of interaction, the energy difference between the states, and the symmetry features of the states.

For electric dipole transitions, for example, the transition probability takes the form:

$$P_{i \rightarrow f} = \frac{2\pi}{\hbar} |\langle f | H_{\text{int}} | i \rangle|^2 \rho(E)$$

where  $P_{i \rightarrow f}$  is the transition probability from the initial state  $|i\rangle$  to the final state  $|f\rangle$ ,  $H_{\text{int}}$  is the interaction Hamiltonian, and  $\rho(E)$  is the density of states (at the energy corresponding to the transition).

Certain transitions are permitted while others are prohibited, and selection criteria assist in assessing the phase of that process. These laws arise from the symmetries of the quantum states pertinent to the transition, including the characteristics of atomic orbitals and the parabolic nature of the electromagnetic field. The selection rules for electric dipole transitions stipulate that the orbital angular momentum quantum number must vary by  $\pm 1$  (i.e.,  $\Delta l = \pm 1$ ), while the total angular momentum quantum number  $j$  may vary by 0 or  $\pm 1$ , except that  $j = 0$  is prohibited from changing by 0. These regulations delineate the permissible transitions and elucidate atomic spectra. The selection principles for transitions and transition probabilities dictate the absorption and emission spectra of atoms and molecules. They allowed us to ascertain the intensities and frequencies of spectral lines, which are crucial for comprehending the behavior of atoms and molecules under diverse settings.

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## Time-Dependent Perturbation Theory

The time-dependent perturbation theory is a mathematical approach to a perturbation of a quantum system by using the time evolution of a quantum particle in the presence of a time-varying external perturbation (e.g. electromagnetic field). This is especially applicable to the situation where the system is not at its ground state and that small perturbations may occur to also excite the system to other energy levels. In its most basic form, one writes down the Hamiltonian of a given system as per the Lorentz and details (unperturbed part,  $H_0$ ) and perturbed part due to interaction outside the system ( $H_2(t)$ ,  $H'(t)$ ) that is dependent on time.  $H = H_1 + H_2 + H_e$  (28) The net Hamiltonian can now be expressed as:

$$H(t) = H_0 + H_2(t)$$

The time evolution of this system is governed by the time-dependent Schrödinger equation:

$$i\hbar \frac{\partial}{\partial t} |\psi(t)\rangle = H(t) |\psi(t)\rangle$$

We then proceed to make several assumptions about the perturbative regime we are in, in order to be able to clean up the equation and express a series in perturbation theory of the solution. For instance, the first-order correction to wave function describes the amplitude for the system to evolve from an initial state to a final state due to action of the perturbation. This is a common approach in studying atomic and molecular transitions driven by electromagnetic radiation, where the perturbing potential is usually the interaction between the system and radiation field. These transition probabilities are calculated using time-dependent perturbation theory: they provide information on rates for absorption/emission processes, something you have looked into in the context of spectra (at a high level).

## Approximation of Born and Oppenheimer



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The Born-Oppenheimer approximation is a method that simplifies the analysis of molecular systems by separating the movements of nuclei and electrons. This approximation relies on the principle that the nuclei of a molecule possess significantly greater mass than the electrons, resulting in their comparatively slower motion. The objective is to decouple nuclear motion from electrical motion, rendering the problem manageable. The Born-Oppenheimer approximation is employed to express the electronic wave function, wherein the locations of the nuclei are fixed, and the nuclei are regarded as classical particles traveling inside the potential created by the electrons. This results in an effective potential energy surface for nuclear motion, which can be utilized to characterize the vibrations and rotations of the molecule. This outcome can serve as a foundation for addressing the time-dependent Schrödinger equation utilizing two distinct coordinates, electronic and nuclear, which culminated in the Born-Oppenheimer approximation, extensively employed in molecular spectroscopy and dynamics. While it significantly simplifies calculations, it is not devoid of limitations. This appears straightforward; nevertheless, it entails certain assumptions, including the premise that the nuclei do not experience quantum effects, which is not universally applicable, particularly in scenarios involving extremely low temperatures or light molecules such as hydrogen. The Born-Oppenheimer approximation is applicable to particular molecular systems with specific potentials where the potential energy surfaces of the nuclei do not intersect, facilitating the separation of electronic and nuclear motions, which results in energy states that often linearly depend on nuclear eigenstates. This concisely delineates the fundamental premise of each theoretical system. To achieve the necessary word count for your paper, please expand upon these subjects and explore specific instances, mathematical derivations, and applications in greater depth.

#### 1.2 Molecular Energy Levels

Molecular energy levels are central to the interaction of molecules with electromagnetic radiation, reactivity in chemical transformations, and the behavior of matter as a function of temperature, as well as physical properties measured by spectroscopy. In a molecule, energy could be stored in different degrees of

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freedom, i.e. translational, rotational, vibration and electronic degrees of freedom, resulting into quantization of energy levels. Of these, the rotational, vibration and electronic energy levels are the most important to understand in the context of molecular spectroscopy, and thus the structure of these transform into the basis for studying molecular behavior in different physical and chemical realms.

### Rotational Energy Levels

Rotational: Rotational energy levels are the energy levels associated with the rotation of a molecule as a whole. In quantum mechanics, the rotational motion of molecules of the molecule is considered as a rigid body system, where the molecule rotates around its center of mass. Such motion is quantized, that is, only certain discrete energy levels are permitted. Molecular rotations can be approximately described by the rigid rotor model, where a molecule is imagined to consist of two vibrating masses, which represent atoms or groups of atoms, and rotate about an axis.

A diatomic molecule can only rotate at specific, quantized rotational energy levels, which are given by:

$$E_J = \frac{J(J+1)\hbar^2}{2I}$$

where:

- $E_J$  is the energy of the rotational level with quantum number  $J$ ,
- $J$  is the rotational quantum number (where  $J=0,1,2,$ )
- $\hbar$  the reduced Planck constant,
- $I$  is the moment of inertia of the molecule and depends on the masses of the atoms and the bond length between them.

For linear molecules the moment of inertia is given by:

$$I = \mu r^2$$



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Here,  $\mu$  represents the decreased mass of the molecule, defined as  $\mu = (m_1 m_2) / (m_1 + m_2)$ , and  $r$  denotes the bond length between the two atoms. Nonetheless, regarding nonlinear molecules, the last statement is not entirely accurate, as the moment of inertia is determined by various bond lengths and the angles between them; still, the underlying concepts remain unchanged! The rotational energy levels, expressed as  $J^2/2I$  in increments of  $J^2/2I$ , indicate that adjacent levels diverge as the quantum number  $J$  escalates. The ground state occurs at  $J=0$ , representing the lowest accessible energy level. The rotational energy levels are pertinent in the microwave area of the electromagnetic spectrum, where molecule rotation can be stimulated by the absorption or emission of microwave radiation. Nonetheless, several compounds exhibit distinct behaviors concerning their rotational energy levels. Consequently, homonuclear diatomic molecules (e.g.,  $O_2$ ) lack a dipole moment and, in contrast to polar molecules (such as  $HCl$  or  $CO$ ), are incapable of interacting with electric dipole radiation, thus unable to absorb or emit microwave radiation. Consequently, the observability of rotational transitions is contingent upon the molecule's dipole moment. Rotational energy levels may be affected by external influences, such as the presence of an electric or magnetic field, or interactions between rotational and other molecular motions, resulting in the Stark and Zeeman effects.

#### Vibrational Energy Levels

Vibrational energy levels relate to the periodic motion of atoms within a molecule, wherein the atoms oscillate about their equilibrium positions. Vibrational energy, like rotational energy, is quantized, allowing only specific discrete energy levels. The vibration of a diatomic molecule can fundamentally be described using the harmonic oscillator approximation, wherein the potential energy as a function of atomic displacement from equilibrium is presumed to be quadratic, much to a spring.

The vibrational energy levels are quantized for a harmonic oscillator:

$$E_v = \left( v + \frac{1}{2} \right) h\nu$$

where:

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- $E_v$  is the energy of the vibrational state with the quantum number  $v$ ,
- $v$  is the vibration quantum number ( $v=0,1,2,\dots$ ),
- $h$  is Planck's constant, and
- $\nu$  is the frequency of the vibration, a function of both the mass of the atoms and the strength of the atomic bonds.

For neighboring vibration levels, the energy separation remains constant and is equivalent to  $h\nu$ . The zero-point energy (the energy at  $v=0$ ) of  $\frac{1}{2}h\nu$  represents the system's minimal energy, as dictated by the Heisenberg uncertainty principle: atoms cannot stay stationary even at absolute zero temperature. However, the harmonic oscillator serves as an approximation that fails to adequately represent real molecule vibrations at elevated energy levels, where anharmonic effects become significant. The energy is represented as an anharmonic oscillator, with parameters accounting for the non-ideal nature of the bond, resulting in energy levels converging as  $v$  grows. In the anharmonic oscillator model, the energy levels may be expressed as:

$$E_v = \left(v + \frac{1}{2}\right) h\nu \left(1 - \frac{v}{\omega_e}\right)$$

where  $\omega_e$  is a parameter which sets the magnitude of the non-harmonics.

Infrared (IR) is the part of the electromagnetic spectrum where vibrational transitions take place, and IR radiation may be absorbed or emitted in the same frequency as the molecular vibration. Thus, the strength of the bond between atoms and the molecular symmetry can affect the intensity of such vibrational transitions. For a vibrating substance, a substance with a dipole moment which changes during vibration interacts strongly with IR radiation than a that does not react with IR radiation when vibration occurs, so due to this we see stronger vibrational absorption spectra of molecule. Furthermore, a single molecule can have multiple vibrational modes, for example stretching, bending, twisting and each type has its quantized levels. These modes have specific frequencies, which correspond to the mass of the atoms in the bonds and the strength of the chemical bonds



themselves. The vibrational modes of polyatomic molecules are often coupled, resulting in more complicated vibrational spectra, with overtones, combination bands, etc.

#### Electronic Energy Levels

Electronic energy levels refer to atomic and molecular configurations and their corresponding electronic states. Regulating Electron Exchange Between Molecules – The electronic structure of a molecule refers to the arrangement of its electrons into different molecular orbital's (MOs), quantized energy levels associated with the MOs. They are usually far higher than rotational or vibration levels and correspond to electronic transitions among various electronic states. The state of the molecule can simply be described as its electronic configuration based on the concept of polymer-orbital theory. The electronic energy levels can be conveniently described by this equation:

$$E_{\text{elec}} = E_{\text{HOMO}} - E_{\text{LUMO}}$$

where:

- $E_{\text{elec}}$  is the electronic energy of the molecule,
- $E_{\text{HOMO}}$  The energy of the highest occupied molecular orbital (HOMO), and
- $E_{\text{LUMO}}$  is the energy of the lowest unoccupied molecular orbital (LUMO).

Molecular electrons can absorb or emit energy during transitions between electronic states, typically within the ultraviolet (UV) or visible parts of the electromagnetic spectrum. These transitions occur when the energy disparity between electronic states corresponds to the energy of the entering or exiting photon. In systems characterized by conjugation, particularly those exhibiting aromatic or prolonged pi-bonding, the electronic transitions are pronounced, yielding distinct UV-Visible absorption spectra. Nonetheless, the arrangement of electronic states in increasingly complex structures, such as polyatomic compounds, can become intricate and result in several electronic transitions. Molecular transitions encompass the shifts



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between singlet and triplet states, as well as transitions among various excited states within the same spin multiplicity. The permissible transitions are governed by selection rules, which determine the energy feasibility of a transition based on angular momentum, parity, and other quantum characteristics. This transition is frequently accompanied with radiation emission, fluorescence, phosphorescence, or other phenomena in molecular excited states, when the molecule emits radiation as it returns to a lower electronic state following excitation. The energy of the emitted light corresponds to the energy differential between the excited state and the ground state in these processes.

**Unit - 3 Microwave Spectroscopy**

Microwave spectroscopy examines the molecular structure, energy levels, and transitions of molecules, particularly focusing on rotational transitions within the microwave area of the electromagnetic spectrum. This type of spectroscopy offers essential information about the physical properties of molecules, such as the distance between atoms, the moment of inertia, and symmetries. Rotational transitions are only significantly affected by both the moment of inertia and the symmetry which is why microwave spectroscopy is well suited for analyzing both gas phase and lighter species. The microwave spectroscopy field is important in numerous sciences including chemistry, physics, and environmental sciences. It offers insights into molecular structural properties, the nature of molecular interactions and even for the identification of molecular species within complex mixtures. Microwave spectroscopy (or EPR spectroscopy) is commonly done in the gas phase, though it can also be performed in condensed phases. The amount of energy associated with microwave transitions corresponds to the rotational movement of the molecules, which can be observed through the resultant spectra with respect to the different characteristics of the molecules. Microwave spectroscopy is general a tool to study rotational spectra, which are the transitions between rotational states of a molecule. Molecules can be categorized in different ways depending on their properties, such as how they behave in response to external conditions such as microwave radiation.

**1.1 Description of molecules**

Microwave spectroscopy divides molecules according to their symmetry and their rotational and dipole moment properties. Microwave spectroscopy, which





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studies the interaction of molecules with microwave radiation, contains rich detail on molecular structure, composition, and dynamics. Molecular symmetry, atomic mass distribution, and molecular permanent dipole moment strongly affect the rotational energy transitions of a whole molecule. These provide a guide that is helpful in understanding the interaction of a molecule with the microwave radiation as well as how spectra of molecule can be observed and analyzed.

#### Linear Molecules

Subsets of molecules in which all atoms are lined up linearly are also referred to as linear molecules. They usually have only one degree of freedom regarding the rotational motion around the axial line of the atoms. Linear molecules may be made up of two or more atoms, chalking the way for diatomic molecules (e.g., oxygen ( $O_2$ ), nitrogen ( $N_2$ ), carbon monoxide ( $CO$ )) as classic examples. Linear molecules have a permanent dipole moment, allowing them to engage with microwave radiation, which is a key signature of linear molecules. In microwave spectroscopy, the transitions that are observed in linear molecules occur when the molecule absorbs or emits radiation that is equivalent to a change between the rotational energy states of the molecule. The microwave spectrum of a linear molecule displays a series of distinct rotational lines resulting from the interaction between its permanent dipole and the electric field of microwave radiation. Rotational levels, quantized for linear molecules, exhibit energy spacing that is contingent upon the molecule's moment of inertia, which is determined by the mass distribution of the atoms and the bond lengths. The rotational transitions of linear molecules are often regulated by a selection rule that necessitates a change in the rotational quantum number ( $J$ ) of the molecule. These molecules typically exhibit a succession of rotational transitions characterized by a clustering that relies on the moment of inertia, which is, in turn, influenced by the rotational constant. If an elongated linear molecule were to rotate, the resulting rotational spectrum would be uncomplicated, defined by distinct lines that correspond to transitions between the molecule's rotational energy levels.

#### Molecules that were bent or polyatomic (non-linear)



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Non-linear molecules commonly referred to as bent or polyatomic molecules, comprise three or more atoms situated in an angular or non-linear configuration. But the rotational spectra of these molecules are more complicated than for the first two types, because they can rotate about many different axes. Non-linear molecules include  $\text{H}_2\text{O}$ ,  $\text{NH}_3$ , and  $\text{CH}_4$ . These molecules generally possess three orthogonal rotational axes, two within the molecular plane and one off the plane. Again, non-linear molecules have more rotational degrees of freedom as compared to that of the linear molecules and hence their microwave spectra are complicated. Note that, for each degree of freedom, we can have a different type of rotational motion and the rotational energy levels will thus be smeared across much more complicated manifolds. However, for non-linear molecules the rotational energy levels depend on three rotational constants related to three different axes of rotation. The spacing's between the rotational levels, and the appearance of the microwave spectrum, are thus governed by these constants. Non-linear molecules stand out among these complexities because they can display vibration transitions that couple to the rotational transitions resulting in further features in the microwave spectrum. Consequently, the analysis of rotational spectra for non-linear molecules involves a more complex characterization of their geometry as well as the coupling between the rotation and vibration motions. However, many features of the microwave spectrum for non-linear molecules give insight into molecular structure, including information about bond lengths and bond angles, as well as the moments of inertia.

### **Spherical Molecules**

Spherical molecules are those which are spherical symmetric. In microwave spectroscopy, these molecules are relatively simple to analyze because they are uniform in all directions. Methane ( $\text{CH}_4$ ) is an example of a spherical molecule high symmetry. Methane is a symmetric tetrahedral molecule in which the hydrogen atoms are arranged symmetrically around a central carbon atom. This symmetry creates an isotropic distribution of mass, which means the rotational properties of the molecule are identical in all directions. The rotational states of spherical molecules are degenerate (the molecule can rotate freely without changing the



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energy). This symmetry makes the analysis of the microwave spectrum easier, as often only one rotational constant need be considered. For spherical molecules, the rotational transitions black and the spectral lines correspond to transitions between quantized rotational levels representative of ( $j = 0, 1, 2, 3 \dots$  (where  $j$  = total momentum quantum number). Hello everyone, I have worked out the rotational spectrum of spherical molecules (typical example: Methane) and it is quite easy to diffuse and hence features will not be complex splitting and other things related to the higher energy level spin states of the molecules. For example, the study of spherical molecules in microwave spectroscopy can provide valuable information about the size of the molecule, moments of inertia, and other structural parameters. Due to this symmetry, spherical molecules are frequently used as model systems to study fundamental mechanisms of rotational spectroscopy and to benchmark theory in molecular physics. They also serve as a theoretical basis for comprehending the behavior of molecules of higher symmetries and more complex structures.

#### Symmetric Top Molecules

The motion of the Symmetric top molecules is more complex. This kind of molecular symmetry gives rise to more complex rotational energy levels than with spherical molecules but is still simpler than the analysis of asymmetric top molecules. Symmetric top (last book table) are molecules like ammonia ( $\text{NH}_3$ ) or methyl fluoride ( $\text{CH}_3\text{F}$ ) there are two equivalent of the rotational axes and one difference. This symmetry leads to the splitting of the rotational energy levels into separate components detectable in the microwave spectrum. The microwave spectrum of symmetric top molecules is also consist of series of lines, with each line corresponding to the transition of the rotational energy levels of a molecule. But due to the symmetry of the molecule, the rotational transitions are more complicated compared to the case of spherical molecules. In the case of the two equivalent axes, for example, the transitions that take place are connected, and split the rotational spectrum into more than one component. These transitions can be understood using the rotational constants associated with each axis of the molecule, as well as the selection rules for rotations which arise from the symmetry of the molecule itself. Symmetric top molecular atmospheres have a bit more complex

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rotational constants and relationship between the quantum states of the molecule. These give a much more complicated pattern of spectra than the spherical ones, but the symmetry makes it easier to interpret than that of asymmetric top molecules. They offer a good platform for studying molecular dynamics, the distribution of rotational energy levels, and interaction of molecules with electromagnetic fields.

### **Asymmetric Top Molecules**

Asymmetric top molecules: The most complicated molecules in aspects of rotational spectroscopy. These are unsymmetrical molecules with respect to the following rotational axes; hence the length of all three rotational axes is different and the rotational constants are also different for this kind of molecule. Asymmetric top molecules are those which have three axes of rotation of which one is more easily rotated than the other two (see image), such as water ( $\text{H}_2\text{O}$ ), hydrogen sulfide ( $\text{H}_2\text{S}$ ) and some large organic molecules. Gunned and his colleagues found that in these molecules, the lack of symmetry makes their microwave spectra more complex and difficult to interpret. Asymmetric top microwave spectra consist of a series of rotational transitions which are more complicated than linear, non-linear or symmetric top molecules. The energy level spectrum for rotations of these molecules is not degenerate, and the transitions between them give rise to uniquely shaped spectra, with lines spread out in frequency and having different intensity and spacing.

**Rotational Components** The rotational states of these large molecules cannot be assigned as simply as it is done for diatomic systems, since the symmetry of these types of molecules is broken due to their large internuclear spacing, thus not allowing the assignment of the rotational states with respect to the three axes of rotation. In contrast, because asymmetric top molecules do not have any equivalent rotational axes, their spectra often show various features: including possible line splitting and intensity alternation that depends on the detailed molecular structure. After all, asymmetric top molecules can give us a lot of information about the molecular structure and molecular rotation dynamics. They become especially significant in the investigation of more sophisticated systems, such as bimolecular, large organic molecules, and another substance with



complicated molecular configurations. Molecular classification into these two **Non-Rigid Rotor and Rigid Rotor Model**

The simplest molecular model can be perfectly represented in microwave spectroscopy by a rigid rotor model. This means that, for this model, a molecule is simply a rigid rotor, no molecules bend or vibrate back and forth as atoms move around with respect to the center of mass of the molecule. The rigid rotor approximation is most frequently used for diatomic molecules, though it can also be applied to some polyatomic molecules with certain constraints.

In the rigid rotor model, the energy conditions associated with rotational motion are described by the relation:

$$E_J = \frac{J(J+1)\hbar^2}{2I}$$

where:

- $E_J$  is the energy of the  $J$  rotational state,
- $J$ = rotational quantum number (an integer value such as 0, 1, 2,...)
- $\hbar^2$  is the reduced Planck constant, where
- $I$ , the moment of inertia of the molecule.

However, real molecules are not perfectly rigid objects: they can vibrate and rotate, and these types of motion influence each other. Therefore, the non-rigid rotor model is commonly used to take into account these interactions. The non-rigid rotor model considers that molecules may slightly deform as they rotate and vibrate. At higher rotational quantum numbers, centrifugal forces come into play leading to some deformations in their structures and breaking the assumption of rigid rotors.

This is based on the modifications of the energy levels due to moving rotors that carry a non-rigid rotor model of centrifugal distortion which adds further corrections to the energy equation. These corrections may be accounted for by

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the introduction of a centrifugal distortion constant, resulting in energy shifts of the rotational levels. For a non-rigid rotor, the energy equation becomes adjusted:

$$E_J = \frac{J(J+1)\hbar^2}{2I} - D_J(J+1)^2$$

$D_J$  is the centrifugal distortion constant. This correction takes into consideration the fact that the moment of inertia increases slightly at the time of rotation of the molecule due to the centrifugal forces experienced by the atoms. For high rotational quantum numbers and for large molecules, the rigid rotor model breaks down, and the non-rigid rotor model becomes crucial. This model takes into account centrifugal distortion effects and gives a better description of rotational energy levels for complex molecules in gas phase.

### Isotopic Substitution Effects

It is the fact that microwave spectroscopy allows one to study the effects of isotopic substitution on molecular spectra. This is when isotopic substitution takes place, that is, one atom is replaced in a given molecule with its isomer, resulting in a change in the mass of the atom without affecting its chemical reactivity. In microwave spectroscopy, isotope substitution primarily influences the moment of inertia, hence affecting the rotational energy levels of the molecule. The moment of inertia of a molecule is dictated by the masses of its constituent atoms and the distances between them. Substituting an atom with one of its isotopes alters the moment of inertia, thereby affecting the rotational energy levels. For example, substituting a hydrogen atom (mass  $m_H$ ) in a diatomic molecule like HCl with its isotope deuterium (mass  $m_D$ ) will result in a distinct moment of inertia due to the greater mass of the deuterium atom. The moment of inertia is defined as follows:

$$I = \bar{r}^2$$

where  $\bar{r}$  is the reduced mass, defined as  $\bar{r} = \frac{m_1 m_2}{m_1 + m_2}$  and  $r$  is the bond length.

Once again, since the moment of inertia is proportional to the mass of the atom, the rotational energy levels will be driven to lower frequencies (longer wavelengths) when the lighter isotope gets substituted by its heavier counterpart. The difference



is especially apparent for isotopic substitutions based on hydrogen ones, where the hydrogen atom is replaced with either deuterium atom (H to D) or tritium atom (H to T), which have much greater difference in the mass between its isotopes. Microwave techniques are used extensively to determine bond lengths by using isotopic substitution, in reaction dynamics and to identify various isotopes in the sample. All types of isotopic substitution are important for probing the physical properties of molecules; this is critical in studies of molecular interactions or in investigations of molecular vibrations in addition to rotational transitions.

### The Stark Effect and Interaction with an External Field

This phenomenon is termed the Stark effect, when the introduction of an external electric field induces the splitting or shifting of a molecule's energy levels. This effect results from the interaction between the molecule's dipole moment and the external electric field. The Stark effect can provide insights about the dipole moment of the molecule, its symmetry, and the impact of external perturbations. The "Stark" effect in microwave spectroscopy is best observed for molecules with a finite permanent dipole moment. The energy levels related to the rotational transitions split or shift when a molecule is introduced to an electric field. The size of the reaction depends on how strong the electric field is and how the dipole moment are oriented to the field. Using perturbation theory, we can calculate the energy shift due to the Stark effect, yielding the following follow expression for energy shift:

$$\Delta E = -\vec{\mu} \cdot \vec{E}$$

where:

- $\Delta E$  the energy shift,
- $\mu$  the dipole moment of the molecule, and
- $E$  the external electric field.

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Molecular properties like dipole moments and polarizabilities can be analyzed with the Stark effect. It is frequently applied for determining dipole moments of gas-phase molecules, for which the field can cause charge separation across the molecule. In addition, the Stark splitting on the rotational spectra can be used to evaluate the distribution of charge in the molecule as well as the geometry of its electronic structure.

**Spin-Spin Interaction: Nuclear and Electron**

**Micro-wave Spectroscopy** The main features of contribution of N/S electron spins in microwave spectroscopy is interaction of N/S spins and their respective contribution to individual electron spin magnetic moment, electron spin resonance spectroscopy etc. Nuclear spin interaction refers to the interaction between the magnetic dipole moment of nuclear spins and the magnetic flux produced by electron spins within the molecule. This leads to a degeneracy splitting of the energy levels, observable in the microwave spectrum. An instance of nuclear and electron spin interaction is the hyperfine interaction, when the energy levels of a molecule are divided due to the coupling between nuclear spin and electron spin. This interaction possesses a hyperfine coupling constant, resulting in further splitting of the molecule's rotational spectrum. In radicals containing unpaired electrons, the interplay between nuclear spin and electron spin significantly influences the overall magnetic characteristics of the molecule. This interaction induces the splitting of energy levels, providing significant insights into the molecule's electronic structure, electron density distribution, and the connection between electron and nuclear spins. This phenomenon is typically investigated using techniques like electron paramagnetic resonance (EPR) spectroscopy, which measures microwave absorption to derive information on spin-spin interactions.

**Multiple-Choice Questions (MCQs)**

- 1. Which of the following best defines spectroscopy?**
  - a. The study of chemical reactions involving radiation





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- b. The study of the interaction between electromagnetic radiation and matter
- c. The study of nuclear reactions in stars
- d. The measurement of temperature using infrared radiation

**2. Electromagnetic radiation consists of:**

- a. Only electric fields
- b. Only magnetic fields
- c. Perpendicular electric and magnetic fields
- d. Only charged particles

**3. Which of the following processes involves the absorption of electromagnetic radiation by a molecule?**

- a. Emission
- b. Reflection
- c. Excitation
- d. Scattering

**4. Which of the following factors contributes to natural line broadening in spectroscopy?**

- a. Doppler effect
- b. Collisional broadening
- c. Heisenberg uncertainty principle
- d. Instrumental limitations

**5. The Born-Oppenheimer approximation assumes that:**

- a. Electrons move much faster than nuclei



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- b. Vibrational and rotational motions occur at the same energy scale
- c. The total energy of a molecule remains constant
- d. The energy levels of atoms do not change

**6. Which type of molecular energy level transitions are associated with microwave spectroscopy?**

- a. Rotational transitions
- b. Vibrational transitions
- c. Electronic transitions
- d. Nuclear transitions

**7. The rigid rotor model is used to describe:**

- a. The electronic energy levels of a molecule
- b. The vibrational motion of atoms in a molecule
- c. The rotational motion of a diatomic molecule
- d. The nuclear spin states of atoms

**8. What is the effect of isotopic substitution on rotational spectra?**

- a. It does not affect the spectra
- b. It changes the moment of inertia and shifts spectral lines
- c. It only affects vibrational spectra
- d. It alters the electronic energy levels

**9. The Stark effect refers to:**

- a. The splitting of spectral lines due to a magnetic field
- b. The splitting of spectral lines due to an external electric field
- c. The broadening of spectral lines due to molecular collisions
- d. The energy shift in nuclei due to relativistic effects



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#### 10. Which of the following is an application of microwave spectroscopy?

- a. Determining molecular geometry
- b. Studying electronic transitions
- c. Measuring nuclear spin interactions
- d. Observing visible light emissions

#### Short Questions

1. Define spectroscopy and explain its importance in chemistry.
2. What are the key properties of electromagnetic radiation?
3. Differentiate between absorption, emission, and transmission of radiation.
4. Explain the concept of natural line width and broadening in spectroscopy.
5. What is the Born-Oppenheimer approximation? Why is it important?
6. Describe the different types of molecular energy levels.
7. Explain the rigid rotor model and its assumptions in microwave spectroscopy.
8. How does isotopic substitution affect the rotational spectrum of a molecule?
9. What is the Stark effect, and how does it influence microwave spectra?
10. List some applications of microwave spectroscopy in scientific research.

#### Long Questions

1. Discuss the interaction of electromagnetic radiation with matter, including absorption, emission, and scattering mechanisms.
2. Explain the fundamental theoretical concepts of spectroscopy, including the uncertainty principle, transition probability, and selection rules.
3. Describe the different molecular energy levels (rotational, vibrational, and electronic) and their corresponding spectroscopic techniques.



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4. Explain the principles of microwave spectroscopy and its application in determining molecular structure.
5. Discuss the rigid rotor model and non-rigid rotor model in microwave spectroscopy.
6. Explain the effects of isotopic substitution on rotational spectra and molecular moment of inertia.
7. Describe the Stark effect and its significance in external field interactions.
8. How does nuclear and electron spin interaction influence microwave spectra?
9. Compare and contrast rotational, vibrational, and electronic spectroscopy in terms of energy transitions.
10. Discuss the major applications of microwave spectroscopy in chemistry, physics, and environmental science.



## ATOMIC, MOLECULAR, AND PHOTOELECTRON SPECTROSCOPY

### Unit-4 The Atomic Spectroscopy

Atomic spectroscopy examines the fundamental interactions between atoms and electromagnetic radiation, elucidating the discrete energy transitions that occur when atoms absorb or emit photons of specific wavelengths, thus offering essential insights into atomic structure, electronic configurations, and quantum mechanical behavior. The fundamental premise involves atomic orbital energy levels—quantized states where electrons are positioned based on quantum mechanics—where transitions between these levels generate distinct spectral lines that act as unique identifiers for each element. The vector representation of angular momentum offers a mathematical framework for comprehending the contributions of orbital angular momentum ( $L$ ) and spin angular momentum ( $S$ ) to atomic properties, as these vectors possess both magnitude and direction in three-dimensional space, facilitating accurate descriptions of electron behavior. Vector coupling schemes, specifically Russell-Saunders ( $LS$ ) coupling for lighter elements and  $j-j$  coupling for heavier elements, elucidate the combination of angular momenta to ascertain the total angular momentum ( $J$ ) of an atom, which directly affects spectral patterns via selection rules and fine structure splitting. Term symbols, shown as  $^{2S+1}L_J$ , succinctly convey an atom's electronic configuration by indicating multiplicity ( $2S+1$ ), orbital angular momentum ( $L$ , denoted by S, P, D, F), and total angular momentum ( $J$ ), thereby allowing spectroscopists to systematically identify and anticipate spectrum transitions. The hydrogen atom spectrum exemplifies the most fundamental and significant instance in atomic spectroscopy, with its energy levels accurately delineated by the Rydberg formula and its spectral series (Lyman, Balmer, Paschen, etc.) corresponding to transitions between designated principal quantum numbers. In contrast, alkali metal spectra introduce further intricacy due to quantum defects that address electron-electron interactions and core penetration

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effects absent in hydrogen. Contemporary applications surpass conventional

**Atomic Orbital's and Their Energy Levels**

Atomic orbital's, spectroscopy, energy levels They correspond to quantized states that the electrons occupy in an atom. Energy levels arise because the electron's energy in the atom is quantized, an effect that occurs due to quantum mechanics. A particle is comparable to the motion of an electron within an atom, where the energy levels of the electron are contingent upon the attraction to the nucleus and the electron's mobility.

In a hydrogen atom, the most fundamental atom, the energy levels are delineated by the Bohr model and can be articulated by the equation:

$$E_n = -\frac{13.6 \text{ eV}}{n^2}$$

where:

- $E_n$  is the energy of the electron at the principal quantum number  $n$ .
- $n$  is the main quantum number (where  $n=1,2,3,\dots$ ).
- 13.6eV is the energy at the lowest quantum-state of hydrogen.

This means that when  $n$  decreases the energy of an electron becomes more negative and when  $n$  increases the energy levels become more close together. The movement of electrons between these energy levels reflects the emission or absorption of some specific wavelengths of electromagnetic radiation, and form the hydrogen atomic spectrum. In many-electron systems, such as multi-electron atoms, energy levels are influenced by both the nucleus and the electron-electron repulsion. It introduces additional complexity to the configuration of the energy levels. In atoms possessing multiple electrons, energy levels can be characterized by: The primary quantum number ( $n$ ), orbital angular momentum quantum number ( $l$ ), magnetic quantum number ( $m_l$ ), and spin quantum number ( $m_s$ ) are essential for comprehending the attributes and behaviors of electrons in atoms. These quantum numbers emerge from the resolution of the Schrödinger equation for an atom,



specifying the quantized energy levels, orbital forms, their orientations, and the intrinsic spin of electrons within the atom. Each quantum number fulfills a distinct role in delineating the features and behavior of the electron, thus establishing a comprehensive characterization of the electron's state in quantum mechanics.

### Introducing the Principal Quantum Number (n)

The first quantum number the principal quantum number (n) It indicates the total amount of energy that the electron carries and is crucial for determining the electron's orbital size and energy. It can take on the positive integer values only (n = 1, 2, 3,...) which correspond to the distance of the electron from the nucleus. The orbital's that are interacting from the nucleus increase up to n. The principal quantum number is connected with the energy of the electron in the atom. For instance, the energy of an electron in a hydrogen atom is inversely proportional to the square of the principal quantum number (n), according to the energy formula:

$$E_n = -\frac{13.6 \text{ eV}}{n^2}$$

where  $E_n$  is the energy of the electron at the  $n^{\text{th}}$  energy level (and  $13.6 \text{ eV} = E_0$ )

Thus, as you increase the value of n, the electron is less tightly bound as the energy becomes less negative, and we say that the electron occupies a higher energy level. For hydrogen like atoms (atoms with a single electron), the principal quantum number gives us a direct relationship between the energies of the atom. But in more complex atom types, with more than two electrons, these electrons cannot be treated separately from each other anymore when it involves their energy being influenced by repulsion in between. Nonetheless, the principal quantum number which reflects the electron's shell number is still the root cause behind the energy and size of an electron's orbital in any atom.

### Orbital angular momentum quantum number l

The second quantum number is the orbital angular momentum quantum number (l) that describes the shape of an electron's orbital. The value of l can take any integer value between 0 to  $n-1$ , where n stands for the principal quantum number.

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For a given value of  $n$ ,  $l$  specifies the type of orbital the electron occupies. For every value of  $l$ , we have a different type of orbital:

- $l=0$ : s orbital (spherical symmetry)
- $l=1$ : p-orbital (dumbbell shaped)
- $l=2$ : d orbital (cloverleaf-shaped)
- $l=3$ : f orbital (complex shapes)

Different amplitude electron density distribution around nucleus is found by these orbital's.

The angular momentum of the electron is also determined by the orbital angular momentum quantum number.  $|L|=mvr$   $|L|=mvr$

$$L = \hbar \sqrt{l(l+1)}$$

where  $L$  denotes the magnitude of the orbital angular momentum and  $\hbar$  represents the decreased Planck constant.  $l$  specifies the angular characteristics of the wave function, dictating the spatial distribution of the electron's probability density. The  $l$  quantum number defines the angular momentum of a particle in the plane and the intricacy of the related orbital's structure; hence, higher  $l$  values indicate greater angular momentum and more complex interactions with external fields. The value of  $l$  also dictates the different potential orientations of the orbital. For any  $l$  value, there are  $2l + 1$  distinct spatial orientations of the orbital, as indicated by the magnetic quantum number  $m_l$ .

The magnetic quantum number ( $m_l$ ) delineates the spatial orientation of the orbital in relation to the applied magnetic field. For a constant value of the orbital angular momentum quantum number  $l$ ,  $m_l$  will assume integer values ranging from  $-l$  to  $+l$ , inclusive of zero. For each value of  $l$ , the magnetic quantum number possesses  $2l + 1$  possibilities. These represent the distinct orientations that an orbital can assume in space. The permissible values of  $m_l$  can be ascertained from the aforementioned expression; for instance, when  $l=1$  (a p orbital),  $m_l$  can assume the values  $-1$ ,  $0$ , and  $+1$ , signifying three potential orientations of a p orbital, as





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illustrated in the image. For  $l=2$  (a d orbital), the values of  $m_l$  can range from  $-2$  to  $+2$ , yielding five potential orientations of the d orbital. Comprehending the magnetic quantum number is essential for grasping the behavior of electrons in magnetic fields. The energy levels of atoms are divided in an external magnetic field based on their magnetic quantum number, a phenomenon referred to as the Zeeman effect. The orbitals, characterized by the  $m_l$  quantum numbers, react variably to the magnetic field, resulting in distinct energy shifts. The effect is often utilized in spectroscopy to analyze the characteristics of atoms and molecules in the presence of a magnetic field. The magnetic quantum number also delineates the degeneracy of energy levels. For instance, orbitals with identical  $l$  values but differing  $m_l$  values exhibit identical behavior (they are degenerate) in the absence of an external magnetic field. The presence of a magnetic field disrupts this degeneracy, eliminating the equivalence of orbitals with differing  $m_l$  eigenvalues at varying energy levels.

#### **The Spin Quantum Number ( $m_s$ )**

The inherent quantum number of spin is referred to as the spin quantum number ( $m_s$ ). The other three quantum numbers define the spatial aspect of the electron's wave function; however, the electron spin quantum number pertains to an inherent characteristic of the electron. Electrons are fundamental particles possessing intrinsic spin. Spin does not represent a physical rotation and does not equate to the actual spinning of an electron; rather, it constitutes a form of angular momentum that contributes to the electron's overall angular momentum. The spin quantum number of an electron possesses two potential values,  $+1/2$  or  $-1/2$ , reflecting the two distinct states of an electron's spin. The spin alignment of these values is commonly designated as "spin-up" and "spin-down," respectively. Identical: The spin quantum number is crucial for defining the magnetic characteristics of electrons and their interactions with particles and fields. Electrons may possess varying spin quantum numbers and interact to create distinct chemical bonds, hence determining the overall atomic or molecule spin state. The spin quantum number is an essential element of the Pauli Exclusion Principle, which asserts that no two electrons in an atom may possess identical sets of four quantum numbers. This forms the foundation for electrons organizing into orbitals and energy levels, with each electron occupying

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its distinct quantum state. When spin is utilized for magnetic properties, the spin quantum number is the determinant of the atom's total magnetic moment, applicable to all atoms except helium. As electron spins can pair (with opposite spins) inside energy levels, when two or more electrons occupy the same energy levels, their spins cancel, leading to a nullification of individual magnetic moments. Conversely, unpaired electrons with parallel spin contribute to the atom's net magnetic properties.

**The Quantum Numbers that Describe the Electron Configuration**

The four quantum numbers ( $n$ ,  $l$ ,  $m_l$ ,  $m_s$ ) collectively delineate the quantum state of the electron within the atom. These quantum numbers delineate the electron arrangement of an atom, specifically its electron configuration within the orbitals. You possess expertise in a certain sequence of electron configuration, progressing from the lowest energy orbital to the highest energy level. The primary quantum number  $n$  defines the overall energy level, whereas the orbital angular momentum quantum number  $l$  specifies the orbital's structure. The magnetic quantum number  $m_l$  delineates the orientation of the orbital, while the spin quantum number  $m_s$  specifies the direction of the spin. Chemists can forecast an atom's binding energy, reactivity, and other qualities that characterize its behavior in chemical processes by evaluating its electron configuration. The valence electrons in the outermost energy levels participate in chemical bonding, affecting the atom's reactivity and the types of bonds it can establish. You study the four quantum numbers: primary quantum number ( $n$ ), orbital angular momentum quantum number ( $l$ ), magnetic quantum number ( $m_l$ ), and spin quantum number ( $m_s$ ), which characterize the quantum state of an electron within an atom. Collectively, these quantum numbers provide a comprehensive and precise characterization of the electron, encompassing its behavior, orbital form, orbital orientation, and spin. Quantum numbers are essential components of atomic structure, spectroscopy, and quantum mechanics. In many-electron atoms, the electron's behavior is influenced not just by the nucleus but also by interactions with other electrons, necessitating the employment of more complex methods such as the Hartree-Fock technique or configuration interaction to approximate the true energy levels. The fine structure



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arises from relativistic effects, while the hyperfine structure results from the interaction between nuclear spin and electron spin. The resultant energy levels are more intricate than those of the hydrogen atom, yet they may still be characterized using sophisticated quantum mechanical models. The energy levels determine the wavelengths of light that atoms may absorb or emit. When an electron transitions between two energy levels, it either absorbs or emits a photon with energy equivalent to the difference between the two levels,  $E_{\text{final}} - E_{\text{initial}}$ , which aids in comprehending atomic spectra discussed in other sections.

This renders their motion a vector representation of moments in atomic spectroscopy. Quantum mechanics posits that moments are vectors, and the overall momentum of a system can be seen as a vector that can be decomposed into components along various spatial directions. The overall momentum of an electron in an atom is represented by the vector sum of its linear and rotational momentum contributions. The angular momentum of an electron orbiting an atom is quantized by quantum numbers, with the orbital angular momentum quantum number denoted as  $l$ . Magnitude of the orbital angular momentum:

$$L = l(l+1)\hbar$$

where  $\hbar$  represents the decreased Planck constant. The angular momentum vector lacks a definite direction, and quantum mechanics integrates the uncertainty principles along with the intrinsic probabilistic characterization of the electron's position and velocity. This picture omits the fact that, alongside orbital angular momentum, there exists the electron's spin angular momentum, represented by the spin quantum number  $s$ . Here,  $j$  is the total angular momentum quantum number, representing the total angular momentum of the electron, which is the vector sum of its orbital angular momentum and spin angular momentum. The total angular momentum vector is defined by its magnitude:

$$J = j(j+1)\hbar$$

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$j$  takes values between  $|l-s|$  and  $l+s$  inclusive and integer steps between. But we will use this in Next sub-section, to calculate the magnetic properties of the atoms, splitting of the energy levels, when an atom is put on an external field.

### Vector Coupling and Term Symbols

Methods for Incorporating Angular Momenta Vector Coupling Computation of Orbital and Spin Angular Momentum Orbital Angular Momentum Spin Angular Momentum Vector Coupling in Quantum Mechanics Vector coupling is a mathematical technique used to amalgamate angular momenta originating from many sources, such as the orbital and spin angular momenta of an electron or the angular momenta of two electrons. Vector coupling principles are employed to ascertain the overall angular momentum of a system and the corresponding term symbols that characterize the electronic state of an atom. In atomic systems, the total angular momentum is the vector sum of the individual angular momenta. The individual angular momenta of electrons in a multi-electron atom are combined to form the total orbital angular momentum  $L$  and total spin angular momentum  $S$ . The total angular momentum  $J$  is derived from the coupling of  $L$  and  $S$ . The permissible values of  $J$  are established through the vector addition of the individual angular momenta:

$$J = L + S, \quad J = |L - S|, \quad J = |L - S| + 1, \quad \dots, \quad J = (L + 1)L/2(2 - L)$$

When the total angular momentum has been established, the atomic electronic configuration can be expressed in terms of a so-called term symbol. For all energy levels we can use the symbolic representation which describes them as such; the all  $S, L, J$ . The following expression represents the term symbol:

$$^{2S+1}L_J$$

where:

- $2S+1$  is the multiplicity (the number of possible spin states),
- Spectroscopic letter ( $S, P, D, F$ , etc.) representing  $L$
- $J$  is the total angular momentum quantum number.



For instance, the term symbol  $2P_{3/2}$  indicates an atom with  $S=\frac{1}{2}$  (a multiplicity of 2), a  $L=1$  (the letter P) and a  $J=\frac{3}{2}$ .

Vector coupling and term symbols characterize atomic states and provide understanding of the energy configuration of atoms. These notions are particularly pertinent when examining the fine structure of atomic spectra, which refers to the splitting of energy levels due to spin-orbit coupling and similar interactions.

### Spectra of Hydrogen and Alkali Metals

Notable and significant instances in atomic spectroscopy include the spectra of hydrogen and alkali metals. These atoms possess electronic configurations that may be succinctly characterized, featuring multiple energy levels and transitions amenable to detailed analysis. The hydrogen atom possesses a single electron, resulting in a spectrum that is less complex than that of multi-electron atoms. The energy levels of hydrogen are quantized and expressed as:

$$E = -\frac{13.6 \text{ eV}}{n^2}$$

For  $n=1,2,3,4$ , the energy levels are:

$$E_1 = -13.6 \text{ eV}, \quad E_2 = -3.4 \text{ eV}, \quad E_3 = -1.51 \text{ eV}, \quad E_4 = -0.85 \text{ eV}, \dots$$

Thus, the rewritten form is:

$$E_n = -\frac{13.6 \text{ eV}}{n^2}, \quad \text{for } n = 1, 2, 3, 4, \dots$$

The emission or absorption of electromagnetic radiation corresponds to transitions between energy levels. The Ryder formula elucidates the wavelengths of transitions that generate the spectral lines observed in the hydrogen spectrum. The hydrogen atom's spectrum has multiple series, including the Lyman series (ultraviolet), the Balmer series (visible), and the Paschen series (infrared), among others. Each series denotes transitions among different energy levels of the hydrogen atom, with the resultant spectral lines produced by electron transitions between these levels. Hydrogen produces a series of crisp spectral lines with recognized

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wavelengths, observable using techniques such as optical emission spectroscopy or absorption spectroscopy. The hydrogen spectrum is sufficiently simple to have been pivotal in atomic theory, aiding our understanding of the spectra of more intricate atomic systems. The spectra of alkali metals, including lithium, sodium, potassium, and rubidium, feature a single electron in their outermost shell, akin to hydrogen. The spectra of alkali metals are more intricate than those of hydrogen due to the presence of a significantly greater number of inner electrons. Fundamentally, the energy levels of alkali metal atoms are governed by the principles of quantum mechanics; however, electron interactions within the atom result in spectral splitting and fine structure.

Certain emission lines are notably renowned, such as the sodium D-lines (at 589 nm), which result from the transition between two energy levels of the sodium atom. The lines originate from transitions between the 3s and 3p orbitals, whereas the fine structure splitting results from spin-orbit coupling. This phenomenon is also observed in the spectra of other alkali metals.

### 3.7 Hyperfine Structure

Alongside the principal transitions, the spectra of alkali metals display hyperfine structure resulting from interactions between nuclear spin and electronic spin. Additional investigations of this fine structure and hyperfine splitting can be conducted utilizing microwave and laser spectroscopic techniques.

**Unit -5 Molecular Spectroscopy****Molecular Energy Levels and Orbital Theory**

Molecular spectroscopy is the study of how electromagnetic radiation interacts with matter, primarily by analysis of the electromagnetic spectrum. Electromagnetic radiation interacts with atoms, molecular molecular. This area of study investigates the ways that molecules interact with radiation within various parts of the electromagnetic spectrum, offering important information on molecular structure, dynamics and interactions. Schematic representation of different types of molecular spectroscopy. In this section we will touch upon molecular energy levels, vibronic transitions, vibrational progressions, Frank-Condon principle, emission spectra and charge transfer spectra and applications. I was searching for the boundaries



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of carbon's corner, and I stumbled across something. Something more than models to sit at each. We can now define molecular energy levels as the discrete energies that molecules can have and which arise from the quantized nature of their electrons, vibrations, and rotations. The quantum mechanical description of atoms and molecules is integral to understanding molecular energy levels. Orbital theory is an important concept for reading the electronic structure of molecules under this consideration. Per molecular orbital theory, atoms combine to form molecular orbitals encompassing the whole molecule. These molecular orbitals are classified as bonding, anti-bonding, and non-bonding orbitals. Security is determined not just by the molecular orbitals that define the bonds between the constituent atoms of the material but also by the electrons involved in each molecular orbital. Transitions between these energy levels transpire when molecules receive or release photons. In basic molecules, chemical bonds, such as covalent bonds in diatomic molecules, originate from the linear combination of atomic orbitals (LCAO), resulting in the formation of bonding and anti-bonding molecular orbitals. This instance, however, features quantized electronic energy levels, with energy corresponding to the difference between bonding and anti-bonding orbitals.

In more intricate compounds, such as polyatomic molecules, the configuration and dimensions of molecular orbitals are more elaborate and can stretch throughout the entire molecule. The spectrum of energy levels in these compounds pertains to electronic, vibrational, and rotational transitions. Electrons shift between various molecular orbitals during electronic transitions, whereas vibrational and rotational transitions pertain to alterations in the locations and orientations of specific atoms inside the molecule. Molecular orbitals represent the energy levels of molecules, which can be graphically depicted as molecular diagrams. Standard diagrams indicate that the lowest energy molecular orbitals are initially populated with electrons, followed by the progressive filling of higher-energy orbitals, in accordance with the Pauli Exclusion Principle and Hund's rule. Electronic transitions are quantified using UV-Vis spectroscopy, while IR and

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microwave spectroscopy pertain to the identification of vibrational and rotational transitions.

**Vibronic Transitions and Electronic Spectra**

Vibronic transitions occur when both electronic and vibrational levels of a molecule are concurrently engaged. This is characteristic of molecular spectroscopy in general, and specifically in UV-Vis spectroscopy. Vibronic transitions occur when an electrical transition coincides with a change in the vibrational state of the molecule. A molecule can be activated by the absorption of light, which is the principle of UV-Vis absorption spectroscopy, wherein an electron absorbs a photon and transitions to a higher electronic state. In actual molecules, vibronic modes interconnect electronic transitions with the vibrational modes of the molecule. Consequently, during an electrical transition, the molecule may concurrently alter its vibrational state. This results in the emergence of peaks or “bands” indicative of the vibrational and electronic energy levels of the molecule in its absorption spectra. The transition between electronic states generates a sequence of molecular electronic spectra. It is important to recognize that each electronic state possesses vibrational levels, which correspond to the potential vibrations of the molecules following electronic excitation, with transitions occurring both between these vibrational levels and among the electronic states. The electrical and vibrational transitions are collectively referred to as vibronic transitions. In a standard UV-Vis spectrum of a molecule, the vibronic transitions manifest as a sequence of closely spaced absorption bands referred to as the vibrational progression. These bands result from the interaction between electrical transitions and the vibrational motion of the molecule. The spacing between these bands signifies the vibrational energy levels, which can yield insights into molecule structure, bond strengths, and additional factors.

**Vibrational Progressions in Excited States**

Energy absorption induces excitation, transitioning molecules from a ground electronic state to an excited electronic state. In addition to this electronic excitation, there may also be a modification in the vibrational state of the molecule,





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resulting in vibrational progressions detected in the excited states. These, frequently referred to as vibrational progressions, are collections of vibrational transitions originating from the excited electronic state of the molecule. Each electronic state of a molecule comprises many vibrational energy levels. The straightforward photon transition to a singular excitation vibrational level is frequently inaccurate, as the molecule is subsequently excited to several vibrational levels. The transitions between vibrational levels generate a vibrational series. This sequence of emissions or absorptions manifests as a succession of peaks in the emission or absorption spectrum, with the energy spacing between peaks corresponding to the difference in vibrational energy. The vibrational progressions can illuminate the molecular dynamics of the system. The vibrational levels involved in excitation, the quantity of these levels in the excited state, and the spacing between them can provide insights about molecule structure and the properties of the relevant excited states, whether electronic or vibrational. In fluorescence spectroscopy, a molecule absorbs energy and transitions to a vibrationally excited electronic state, then relaxing to a lower energy electronic state while emitting a photon. The emission spectrum will exhibit a vibrational progression, as the emitted photon can shift between several vibrational states. These phenomena is crucial for comprehending the relaxation of molecules from an excited electronic state to the ground state.

#### Frank-Condon Principle

The v-distribution of vibronic transitions is characterized by the Frank-Condon principle. It signifies that electronic transitions occur almost instantaneously in relation to nuclear action. This indicates that the locations of the nuclei in the molecule remain relatively unchanged during an electronic transition. Consequently, the likelihood of a vibronic transition between two electronic states is dictated by the characteristics of the vibrational wave functions of both states. According to the Franck-Condon principle, the strength of a vibronic transition is proportional to the square of the vibrational overlap integral between the beginning and final vibrational wave functions. This overlap is dictated by the spatial arrangement of nuclei in the ground and excited electronic states. This results in transitions between the two states that include minor displacements of the nuclei being far more



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probable. Consequently, the Frank-Condon principle produces a characteristic pattern in molecular spectra. In the excited state, transitions to higher vibrational levels often exhibit lesser strength than those to lower vibrational levels, resulting in a succession of spectral peaks corresponding to various vibrational excitations. This notion is crucial for elucidating the intensity distribution of peaks in molecular spectra and serves as the foundation for absorption and emission spectra. The Franck-Condon principle elucidates the structure of the bands of a spectrum. Due to the elevated probability of transitions involving minimal nuclear displacement, the bands of a molecular spectrum generally exhibit a distinctive “progression” of peaks, with the most prominent peak corresponding to a zero-vibration transition. This sequence of occurrences assists scientists in collecting information about the vibrational behavior of the molecule and the characteristics of the electronic states.

It provides valuable insights into how molecules behave when they absorb energy and return to their ground state, as well as the emission spectra. Fluorescence describes the light emission following the absorption of photons and is a helpful tool for determination of molecular electronic, vibrational, and rotational states. Emission occurs by two process radiative (where photon is produced) and non radiative (where photon is not produced). Both processes describe ways in which a molecule releases the energy it had previously absorbed, but their difference rests in the way this energy is dispersed by the emission of light or otherwise, without any emission of photons. Two main decay mechanisms are radiative (As in luminescent processes) and non-radiative (As in internal conversion or vibrational relaxation) and both processes are required to explain a range of optical properties, Plasmon phenomena as well as single molecule behavior based on its excitation state. Radioactive decay is an important phenomenon in the investigation of molecular behavior, and plays a crucial role in the field of spectroscopy. It describes the step of an atom from a high energy state to a lower energy state while releasing a photon. The photon is the energy lost from the molecule in the transition. Important part of emission spectra, which helps in studying electronic, vibration and rotational states. Radioactive decay plays a crucial role in techniques like fluorescence and phosphorescence, as the radiation emitted contains important molecular structural and dynamic information. Radioactive decay of a molecule is when the molecule



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absorbs the energy (usually from some light photon) and gets excited upwards to some higher energy state. In the next stage of the process, the molecule relaxes back down to its ground state or a state of lower energy (possibly emitting some phonon energy) and releases the energy difference between the two states as a photon of light. The energy of the emitted photon matches the difference in energy between the excited state and the lower energy state the molecule relaxes to. Fluorescence and phosphorescence are the most common examples of the radioactive decay process, which can be either a transition between energy or free electronic states. Each one of these mechanisms has its own characteristics regarding the timescale, energies involved and type of transition, and therefore is a profound tool for the study of molecular properties.

#### **Emission Spectra: Radiative and Non-Radiative Decay**

Radiative decay is an essential mechanism in molecular spectroscopy. The radiation emitted by the emission spectrum provides insights on the electronic and vibrational characteristics of molecules, including their chemical composition, molecular structure, and molecular dynamics. The particulars of radioactive decay depend on the energy levels involved, the electronic states of the molecule, and whether the molecule exists in an isolated or organized environment. The two primary forms of radioactive decay processes are fluorescence and phosphorescence. Both represent kinds of electronic relaxation resulting in light emission; yet, they significantly differ in their specific characteristics concerning excited states and emission kinetics.

#### **Fluorescence**

One of the most common forms of radioactive decay is fluorescence, which refers to the process by which a molecule in an excited electronic state transitions to a lower energy state by emitting a photon. The fluorescence process is nearly immediate, occurring on the range of nanoseconds to microseconds, making it one of the most prevalent tools in molecular and biological research. Fluorescence, akin to other photo physical phenomena, can be delineated in several steps crucial for understanding the mechanism of emitted light. Upon the absorption of a photon, a molecule transitions from its ground state to an excited state. This is



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typically, though not invariably, an electronic transition in which an electron within the molecule is elevated to a higher-energy state. Subsequently, the remainder will occur as the molecule becomes agitated and subsequently relaxes to a lower energy state. Prior to emitting a photon, the molecule frequently experiences a process termed vibrational relaxation. At this step, the molecule commences the dissipation of a portion of its tremendous energy to the surroundings in the form of heat. The entire energy loss results in a diminished vibrational energy level in the molecule's excited electronic state. Subsequent to vibrational relaxation, the molecule emits a photon as it transitions to a lower energy electronic state, typically the ground electronic state or a proximate vibrational state inside the ground state. The emission is characterized as an energy transition, guaranteeing that the emitted photon possesses lower energy (and hence a longer wavelength) than the absorbed incident photon. The energy disparity is referred to as the Stokes shift and is a defining characteristic of fluorescence. Energy is wasted as heat via vibrational relaxation prior to photon emission, resulting in the Stokes shift. Fluorescence is a crucial instrument in various scientific domains, including chemical analysis, biological imaging, and materials science. This technique offers excellent sensitivity and a very straightforward experimental configuration, rendering it an essential instrument for exploring molecular dynamics in solutions, biological systems, or intricate materials. Fluorescence serves as a crucial instrument for imaging and diagnostics, such as fluorescence microscopy, which visualizes cellular structures and monitors molecular interactions in real-time. Fluorescence spectroscopy detects and analyzes emitted light to acquire information regarding the molecular characteristics of the sample. The fluorescence emission spectrum provides insights into the involved electronic states, the energy differential between the ground and excited states, and the molecular dynamics. The fluorescence peaks in the emission spectrum can fluctuate in both strength and position according to several factors, including solvent effects, temperature, and molecular interactions, hence rendering fluorescence spectroscopy a potent method for investigating molecular environments.

## Phosphorescence



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Another type of radiative decay is phosphorescence, which differs from fluorescence in both the excited state nature as well as in the timescale of emission. The excited molecule in phosphorescence goes through a transition from an excited singlet state to a triplet state, which produces a considerably slower light-emission process. The triplet state is at lower energy than the singlet state, but the pathway between the two is “forbidden” according to quantum mechanics, which means the transition happens with much lower probability and on a much slower timescale. Due to this forbidden transition, the light emission of phosphorescence can last much longer than fluorescence, from microseconds to even hours. The long lifetime of phosphorescence makes it especially advantageous for applications that require a long-lived emission, such as in glow-in-the-dark materials, bioluminescent) and certain biological imaging, and investigation of molecular dynamics over longer timescales. Phosphorescence is a photoluminescence process related to fluorescence in which a photon is absorbed and emitted with a delay due to forbidden transitions between energy states. In the case of fluorescence, the molecule changes from one singlet state (an electronic state with paired spins of the electrons) to another while in phosphorescence, it goes from a singlet state to a triplet state (an electronic state with unpaired spins of the electrons). The triplet state manifested due to spin-forbidden transitions and must also change the spin orientation, thus resulting in sluggish relaxation. The mechanism of transition differs between phosphorescence and fluorescence; thus, the emission process is slower than that in fluorescence. In their emission spectra phosphorescent molecules are similar to fluorescent molecules, the main difference being the time scale of the emission. Since the transition to the triplet state is energetically less favorable, phosphorescent emissions generally happen at a lower energy (longer wavelength) than fluorescence emissions. The performance of phosphorescent emission such as the precise wavelength and intensity can be exploited to infer the energy levels and electronic structure of the molecule, as well as the environmental context where the molecule is situated. Studies of the interaction of two molecules, the photon and its phosphorescence implications, and development of luminescent materials for use in OLEDs, sensor technologies, and phosphorescent probes in biological systems are common applications of phosphorescence.



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Phosphorescence can be especially useful in some detection strategies where it allows for delayed light emission providing higher sensitivity and resolution (i.e., the longer-lived nature of phosphorescence as compared to fluorescence).

**Those emission spectra arise from radiative decay.**

The emission spectrum resulting from radioactive decay typically produces multiple lines or bands. These denote numerous transitions between the vibrational or rotational levels of elevated electronic states and the molecule's ground state. Our emphasis was on electronic transitions; nevertheless, the energy levels involved are contingent upon the electronic state of the molecule as well as its vibrational and rotational states. Emission spectra are typically analyzed based on the energy differential between the molecule's beginning and final states. The emission spectrum of molecules transiting between electronic states reveals details on the molecule's electronic structure, the energy necessary to elevate the molecule to a higher energy state, and the energy emitted when it reverts to the ground state. The intricate complexity of the emission spectrum arises from the vibrational and rotational levels involved in the transitions. The emission spectra of fluorescent compounds generally feature peaks that correspond to transitions among several vibrational energy levels in the ground electronic state. The peaks are arranged based on the vibrational frequencies of the molecule, providing insights into its structure and behavior. Phosphorescence spectra provide insight into triplet states and their associated transition mechanisms.

**Non-Radiative Decay**

This energy can be released non-radiatively, wherein the molecule in an excited state ( $S_1$  for singlet or  $T_1$  for triplet) dissipates its energy without photon emission. The molecule does not emit energy in the form of light; instead of radiating energy as light, it discharges surplus heat through several methods that do not generate electromagnetic radiation. Non-radiative processes are essential for understanding energy loss pathways and the overall efficiency of energy transfer; thus, they are significant in any system that aims to avoid light production. The primary mechanisms of non-radiative decay encompass various processes, notably internal conversion (IC) and vibrational relaxation (VR). Radiative decay entails the



conversion of electronic energy into light, whereas these processes require the transformation of electronic energy into thermal energy or the redistribution of vibrational energy among molecular degrees of freedom.

#### **Internal Conversion (IC)**

Internal conversion is a non-radiative mechanism wherein an excited electronic state of a molecule often transitions to a lower electronic state without the emission of a photon. This mechanism is often mediated by interactions with other molecular states, such as the coupling of electronic and vibrational vibrations inside the molecule. This allows the molecule to expel excess energy by transferring it to its vibrational modes, facilitating a shift to a lower electronic state and thereby dissipating energy as heat. Internal conversion is a rapid process that transpires on the femtosecond to picosecond period. A system in which the energy of the excited molecule is not effectively emitted as fluorescence or phosphorescence becomes particularly significant. Internal conversion efficiency is determined by vibrational coupling between electronic states and the presence of a relaxation pathway connecting these states. Numerous internal conversion molecules facilitate the system's relaxation into the ground electronic state, which is pivotal in influencing molecular photophysics.

#### **Vibrational Relaxation (VR)**

A significant non-radiative decay mechanism is vibrational relaxation. This phenomenon is termed vibrational relaxation, referring to the process wherein a molecule in an excited state dissipates extra vibrational energy through collisions with adjacent molecules or atoms, thereby transferring its energy to them. The surplus energy is released as heat, causing the molecule to transition to the ground vibrational state within the same electronic energy level. Vibrational relaxation is a solely thermal mechanism and is a primary method via which stimulated molecules revert to equilibrium. Vibrational relaxation generally occurs over a longer timescale than internal conversion (also in the picosecond to nanosecond range) and, contingent upon the environment and specific chemical system, often exceeds this duration. The significance is apparent in assessing the efficiency of



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photochemical reactions and the dynamics of molecules in condensed phases, where molecular collisions are considerably more probable. Vibrational relaxation is a crucial notion in the dissipation of excess energy in big systems such as proteins and nucleic acids, facilitating the system's energy equilibrium by dissipating the absorbed energy, hence enabling the molecule to stabilize in a lower energy state.

**Comparison between Radiative and Non-Radiative decay**

Radiative and non-radiative decay are fundamental mechanisms that facilitate the relaxation of agitated molecules. Radiative decay refers to the emission of photons and underpins the phenomena of fluorescence and phosphorescence, while non-radiative decay entails the dissipation of excess energy as heat, exemplified by internal conversion and vibrational relaxation. The processes of interest in this example are dictated by the molecular type, the energy levels engaged, and the environmental variables. Radiative decay process when the emitted light type is noteworthy. This dynamic is crucial in scientific fields such as fluorescence-based imaging techniques, gadgets, and spectroscopic analysis. Conversely, non-radiative decay mechanisms are essential to examine in scenarios characterized by poor emission quantum yields or where emission is not the preferred, competing process. In certain molecular systems, such as organic solar cells or light-emitting diodes (LEDs), the efficacy of radiative decay is crucial to the device's performance. The goal of these systems is to optimize radiative output while reducing losses from non-radiative processes. Conversely, in non-radiative decay processes, such as internal conversion and vibrational relaxation, these processes become significant, wherein the conversion of energy to heat may constitute the fundamental objective of a system.

**Charge Transfer Spectra of Transition Metal Complexes**

Charge transfer (CT) spectra in transition metal complexes occur when an electron transitions between the metal center and the ligand, leading to pronounced absorption bands in the UV-Vis spectrum. The transitions are categorized as metal-to-ligand charge transfer (MLCT) and ligand-to-metal charge transfer (LMCT). MLCT transpires when an electron is elevated from a metal-centered





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orbital (usually a d-orbital) to an unoccupied or partially occupied ligand-centered orbital, frequently observed in complexes with ligands like bipyridine, phenanthroline, or phosphines. This transition is evident in metal complexes where the metal exhibits a low oxidation state and the ligand possesses a strong electron affinity, as observed in Ru(II) or Fe(II) complexes. Conversely, LMCT transitions entail electron transfer from a populated ligand orbital to an unoccupied or partially occupied metal d-orbital, commonly seen in complexes with metals in elevated oxidation states and ligands that are readily oxidizable, such as  $\text{MnO}_4^{2-}$  or  $\text{CrO}_4^{2-}$ . The energy necessary for charge transfer is contingent upon the ligand field, the metal's oxidation state, and the characteristics of the donor and acceptor orbitals. CT transitions generally exhibit more intensity than d-d transitions owing to their elevated likelihood according to electronic selection rules. The placement of CT bands in the spectrum elucidates the electronic characteristics of metal-ligand interactions and aids in ascertaining oxidation states and coordination environments in complexes. LMCT transitions, frequently seen in oxoanions and metal-halide complexes, result in vivid hues due to significant absorptions in the visible spectrum. MLCT transitions are essential in photophysical and photochemical applications, including solar energy conversion and photocatalysis. In mixed-valence complexes, intervalence charge transfer (IVCT) may transpire, facilitating the movement of an electron between metal centers, resulting in extensive absorptions within the near-infrared spectrum. CT spectra analysis is an essential technique in spectroscopy for elucidating intricate electronic structures and bonding in transition metal complexes. The capacity to differentiate CT bands from d-d transitions enhances comprehension of redox characteristics and reactivity, serving a vital function in domains such as coordination chemistry, catalysis, and materials research.

#### Applications of Molecular Spectroscopy

Molecular spectroscopy is a potent analytical method employed in diverse scientific and industrial fields to examine molecular structures, interactions, and compositions. A vital application is in chemical analysis and identification, where techniques like Infrared (IR) and Raman spectroscopy facilitate the determination of functional groups in organic and inorganic molecules. This is crucial in medicines,



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forensic science, and quality assurance in chemical manufacture. Molecular spectroscopy serves a crucial role in environmental monitoring by facilitating the identification of pollutants, including greenhouse gases, volatile organic compounds, and heavy metal contaminants in air, water, and soil. Methods such as UV-Vis and fluorescence spectroscopy are extensively utilized in the examination of environmental contaminants and their impacts on ecosystems. In the medical and biomedical domains, spectroscopy is essential for disease diagnosis and pharmaceutical development. Near-infrared spectroscopy (NIRS) is employed in non-invasive medical imaging, whereas nuclear magnetic resonance (NMR) spectroscopy is crucial for metabolic profile and biomarker identification in illnesses including cancer and neurological diseases. Another notable application is in food science and agriculture, where spectroscopy is utilized to evaluate food quality, identify adulterants, and examine nutrient composition. Methods such as Fourier-transform infrared (FTIR) and Raman spectroscopy facilitate the assurance of food safety and authenticity. Molecular spectroscopy is essential in the pharmaceutical sector for drug formulation, stability analysis, and regulatory adherence, guaranteeing that medications satisfy rigorous quality criteria prior to market release. Astronomy and space science utilize molecular spectroscopy to examine the composition of celestial bodies, interstellar materials, and atmospheric phenomena. This facilitates comprehension of cosmic occurrences and the pursuit of extraterrestrial life. Molecular spectroscopy is essential in material science and nanotechnology, aiding in the characterization of novel materials, polymers, and nanostructures, hence facilitating progress in electronics, sensors, and energy storage devices. In forensic science, spectroscopic techniques are employed for material identification, counterfeit detection, and crime scene analysis. Raman and mass spectrometry techniques yield accurate molecular signatures for forensic purposes. Molecular spectroscopy is widely employed in petrochemical industries for hydrocarbon analysis, crude oil characterization, and the monitoring of refinery activities. Energy research is enhanced by spectroscopy, especially in the examination of photovoltaic materials for solar cells, catalysis, and fouling. Ongoing improvements in spectroscopic techniques are expanding its applications, fostering creativity across several scientific disciplines and sectors, thereby



rendering it an essential instrument for contemporary research and technological advancement.

#### **Unit- 6 Photoelectron Spectroscopy (PES)**

Nonetheless, you remain uninformed about Photoelectron Spectroscopy (PES), a remarkable scientific technique that enables the measurement of binding energies of electrons in atoms and molecules. PES is employed to irradiate a sample with photons and quantify the energy of individual electrons upon their ejection from the sample, providing critical insights into the electronic structure, chemical content, and bonding characteristics of the material. It is highly beneficial in fields such as surface science, materials science, and chemistry, which necessitate an understanding of the electronic states of atoms and molecules.

#### **The Basics of the Photoelectric Effect**

The photoelectric phenomenon A phenomenon when electrons are emitted from a substance following exposure to electromagnetic radiation. Photoelectron Spectroscopy (PES) necessitates comprehension of the fundamental principles of the photoelectric effect. The phenomena were initially observed by Heinrich Hertz in 1887, but it gained widespread recognition following Albert Einstein's explanation in 1905, for which he was awarded the Nobel Prize in Physics in 1921. PES operates by hitting a sample with high-energy photons, often X-rays or ultraviolet light. The energy of a photon must exceed a threshold known as the work function to dislodge electrons from it. The energy of the expelled electron is measured, which correlates with the binding energy of the electron within the material. The kinetic energy  $E_{\text{kin}}$  of the released electron is articulated by Einstein's photoelectric equation:

$$E_{\text{kin}} = h\nu - \phi$$

**Where:**

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- $E_{\text{kin}}$ : kinetic energy of the emitted electron;
- $h$  is Planck's constant,
- $\nu$  = frequency of the incident photon,
- $\phi$  the work function, the minimum energy needed to leave an electron on the surface of the material.

By measuring the kinetic energy of electrons released upon the absorption of incident photons with known energy, one can compute the binding energy of each electron within the atom or molecule, therefore elucidating the electronic structure. The energy of the ejected electron signifies the amount of energy required to extricate it from the binding condition. The binding energies correlate to certain electron orbitals, facilitating the identification of chemical elements and the examination of their electronic states.

#### Koopman's Theorem and the Ionization Process

In photoelectron spectroscopy, a photon irradiates the sample, resulting in the absorption of energy that causes the ionization of the target atom or molecule, specifically the ejection of an electron. This pertains to quantum physics, and the ionization energy corresponds to the energy required to ionize a bound electron. To an occupied molecular orbital

(MO). One example of a fundamental component of PES is Koopman's theorem, which provides an initial approximation of a molecule's ionization energy. Koopman's theorem, developed by B. Koopmans in 1933, posits that the configurations of the remaining electrons remain identical when an electron is removed from a molecule. The theorem states that for a molecular Hamiltonian, the ionization energy of an electron corresponds to the negative value of the energy of that electron's orbital within the molecule, as described by Hartree-Fock theory.



Koopman's theorem mathematically suggests that the binding energy ( $E_{\text{binding}}$ ) of an electron in a specific orbital will be approximately:

$$E_{\text{binding}} = -\epsilon_i$$

Where:

- $\epsilon_i$  is the energy of the molecular orbital of the removed electron, is obtained from quantum mechanical calculations.

Koopmans's theorem serves as a valuable framework for comprehending potential energy surface (PES) spectra, especially for simple molecules or atoms with established electron configurations. Nonetheless, for intricate compounds where electron correlation effects are significant, the theorem's accuracy may be limited, as the loss of a single electron can affect the distribution of the remaining electrons. Koopman's theorem, despite its limitations, is essential for interpreting the results of PES and serves as a valuable first-order basis for theoretical analyses of ionization energies.

### Structural Interpretation of PES of Simple Molecules

In particular, the interpretation of PES spectra entails inferring the binding energy of electrons in specific chemical orbitals from the position and intensity of each peak in the spectrum. In PES studies, the energy of incident photons is often established far beyond the threshold required to ionize core-level electrons that are firmly bound by the nucleus. The PES spectra of simple molecules are very straightforward to interpret. The peaks signify electrons from various orbitals, with the binding energy of each peak corresponding to the energy required to ionize an electron in each orbital. The peaks in the PES spectrum are generally categorized based on the type of orbital from which the electron emanates (i.e., s-orbitals, p-orbitals, or d-orbitals). A typical PES spectrum of a simple molecule displays peaks that have been meticulously allocated to the

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chemical orbitals from where they were emitted. Core-level peaks denote the ionization energies of electrons in inner orbitals (e.g., 1s, 2s, and 2p), while valence-level peaks relate to the ionization energies of electrons in outer molecular orbitals. The relative intensities and positions of these peaks can be examined to derive essential information regarding the molecule electronic structure. The relative intensities of the peaks indicate the number of electrons in a specific orbital, while their binding energy values reflect the contributions from the atomic composition, bonding, or chemical environment of the molecule. The PES spectrum for small molecules can exhibit distinct peaks, with spectral separation indicative of several electronic states. The quantity of electronic states and the interactions among electrons markedly escalate for more intricate molecules, potentially doubling the complexity of the PES spectrum, resulting in overlapping peaks and more intricate features.

**Electron Spectroscopy for Chemical Analysis (ESCA)**

ESCA, also known as X-ray Photoelectron Spectroscopy (XPS), is a prevalent surface analysis approach grounded in photoelectron spectroscopy. X-ray photoelectron spectroscopy (ESCA) offers comprehensive insights into surface composition, chemical bonding, and electronic states by analyzing the kinetic energy of electrons ejected from the sample's surface during X-ray exposure. In ESCA, a sample is subjected to X-ray radiation, and the emitted photoelectrons are collected and evaluated. By quantifying the energies of the photoelectrons, one can ascertain their binding energies and get insights into the oxidation states of the chemical elements. ESCA is particularly effective for surface analysis as the emitted photoelectrons predominantly originate from the uppermost layers of the material, typically within the top 1-10 nm. ESCA offers a significant advantage by delivering chemical information regarding the material's surface. The precise binding energy of the photoelectrons is contingent upon the



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element's identification and the atom's chemical surroundings. An element in an alternate oxidation state or bonded to a different atom will exhibit a somewhat distinct binding energy. ESCA is responsive to variations in chemical bonding and may differentiate between the chemical states of an element.

#### ESCA Center: Chemical Information

The chemical information gained by ESCA relies mainly on the examination of the binding energies of core-level electrons. A photoelectron = E photon - I

The photoelectron energy is given by:

$$E_{\text{binding}} = h\nu - E_{\text{kin}} - \phi$$

Where:

- $E_{\text{binding}}$  the binding energy of the electron,
- $h\nu$  – is the energy of the incoming X-ray,
- $E_{\text{kin}}$  represents the measured kinetic energy of the emitted electron,
- $\phi$  is the work function.

The sample's chemical composition can be determined by measuring the binding energy of certain electrons from designated core levels (1s, 2p, etc.). Consequently, peaks corresponding to various elements will manifest at their distinctive binding energies, facilitating their qualitative identification. The precise structure of the PES peaks provides more chemical information. The binding energy of an electron is determined by both the atom's identity and the chemical environment surrounding it. An atom in a metal, oxide, or organic compound has a marginally altered binding energy as a result of its local environment. This great resolution facilitates the examination of oxidation states, chemical bonding, and molecular interactions at the atomic level. While predominantly utilized for elemental analysis, ESCA investigates the chemical shifts that arise when atoms exist in varying chemical environments. By associating these changes

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with the bonding and coordination of atoms within the material, we acquire significant insights into the surface chemistry of materials.

**Auger electron spectroscopy (AES)**

Auger electron spectroscopy (AES) is an additional surface analysis technique associated with photoelectron spectroscopy (PES) and electron spectroscopy for chemical analysis (ESCA). This method entails the identification of expelled electrons from the material resulting from the Auger effect during the de-excitation of the atom following the ejection of a core-level electron. The Auger effect involves the ejection of an inner-shell electron from an atom, resulting in the atom being in an excited state. This additional energy is transferred to another electron within the atom, compelling its expulsion. The energy of released Auger electrons is characteristic of the element and its chemical state. AES is frequently employed to investigate the surface chemistry of samples, particularly when the analytical surface is susceptible to sputtering from surface contaminants. PES and ESCA focus on the binding energies of core-level electrons, while Auger electron spectroscopy (AES) provides complementary information by examining the energies of Auger electrons emitted from the material during the Auger process. AES is very advantageous for analyzing the surface composition of materials and is extensively utilized in materials science, semiconductors, and catalysis.

**Multiple-Choice Questions (MCQs)**

1. Which of the following determines the energy levels in an atom?
  - a) Proton number
  - b) Electron configuration
  - c) Neutron number
  - d) Atomic mass
2. In atomic spectroscopy, the term symbols are derived using:





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- a) Energy differences
- b) Vector coupling of angular momenta
- c) Orbital shapes
- d) Spectral lines

**3. The Frank-Condon principle explains:**

- a) Electronic transitions in atoms
- b) Vibrational structure in electronic spectra
- c) Rotational transitions in molecules
- d) Molecular orbital overlap

**4. Which of the following elements exhibits a hydrogen-like spectrum?**

- a) Oxygen
- b) Helium+
- c) Carbon
- d) Sodium

**5. Molecular spectroscopy provides information about:**

- a) Nuclear structure
- b) Electronic and vibrational states
- c) Crystal defects
- d) Magnetic fields

**6. What does Koopman's theorem state in photoelectron spectroscopy?**

- a) The ionization energy equals the negative of the orbital energy
- b) The photoelectron spectrum depends on nuclear charge



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- c) The intensity of peaks is independent of the molecular structure
- d) The ionization energy is equal to the kinetic energy of the emitted electron

**7. Which type of decay process does not involve photon emission?**

- a) Fluorescence
- b) Phosphorescence
- c) Non-radiative decay
- d) Spontaneous emission

**8. Electron Spectroscopy for Chemical Analysis (ESCA) is primarily used for:**

- a) Determining molecular weight
- b) Studying oxidation states and chemical bonding
- c) Detecting radioactivity
- d) Measuring magnetic moments

**9. Which of the following factors affects the spectral shift in molecular spectroscopy?**

- a) Temperature
- b) Pressure
- c) Molecular environment
- d) All of the above

**10. Auger Electron Spectroscopy (AES) is based on:**

- a) Absorption of X-rays
- b) Secondary electron emission



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- c) Energy transfer between electrons
- d) Ionization of core electrons

#### Short Answer Questions

1. What are atomic orbitals, and how do they relate to atomic energy levels?
2. Explain the concept of vector coupling in atomic spectroscopy.
3. What are term symbols, and how are they derived?
4. Describe the significance of vibrational progressions in molecular spectra.
5. How does the Frank-Condon principle influence molecular electronic transitions?
6. What is the primary difference between emission and absorption spectroscopy?
7. Define Koopman's theorem and its application in photoelectron spectroscopy.
8. What kind of chemical information can be obtained from ESCA?
9. How does charge transfer spectroscopy apply to transition metal complexes?
10. What is the basic principle of Auger Electron Spectroscopy?

#### Long Answer Questions

1. Explain the energy level structure of atomic orbitals and its significance in atomic spectroscopy.
2. Discuss vector representation of momenta in atomic spectroscopy and its role in determining atomic properties.
3. Describe in detail the electronic spectra of hydrogen and alkali metals and compare their spectral features.



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4. Explain the molecular orbital theory and how it helps understand molecular spectroscopy.
5. Describe the principles of vibronic transitions and their role in molecular electronic spectra.
6. Discuss the Frank-Condon principle and its implications in molecular spectroscopy.
7. Explain the process of radiative and non-radiative decay and their significance in emission spectroscopy.
8. Describe the principles of photoelectron spectroscopy and its applications in chemical analysis.
9. Discuss Electron Spectroscopy for Chemical Analysis (ESCA) and the types of chemical information it provides.
10. Explain Auger Electron Spectroscopy (AES), its working principle, and its applications in material science.



#### Module -3

### INFRARED SPECTROSCOPY

#### Unit -7 Fundamentals of Infrared Spectroscopy

Infrared Spectroscopy (IR) is a technique for measuring the vibrational mass of molecules when they absorb infrared. Useful insights on a substance's molecular structure, functional groups, and bonding can be gleaned from this. Applications of Infrared Spectroscopy IR spectroscopy provides useful data about molecular vibrations and chemical compounds in chemistry, biochemistry and materials science. The underlying principles of IR spectroscopy is among the most important but also less understood topics in chemistry because molecular vibrations, energy quantization, and the potential energy surfaces that characterize these vibrations are all involved. The main theoretical background which is critical to understand IR spectroscopy which includes linear harmonic oscillator model, zero- point energy, force constants, anharmonicity, and Morse potential diagram will be described in this section.

#### Model of a Linear Harmonic Oscillator

This is called the Linear Harmonic Oscillator (LHO) Model– a simple model of a vibrating molecule. In IR spectroscopy, the model describes how the atoms in a molecule move relative to one another as they vibrate. In the LHO model, the restoring force on a vibrating molecule is based on the idea that the displacement of the atoms from the equilibrium position is directly related to the force acting on them. It is similar to the action of a mass attached to a spring, which is pushed or pulled, the spring providing a restoring force. The LHO model has a special role in elucidating the foundation physics of molecular vibrations.

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The potential energy  $V$  for a harmonic oscillator according to classical mechanics is given by:

$$V(x) = \frac{1}{2}kx^2$$

Where:

- $V(x)$  is a potential energy as a function of the displacement  $x$ ,
- $k$  is the force constant, which shows the stiffness of the bond between atoms,
- $x$  is the displacement of the atoms from their equilibrium position.

In our model, the atoms vibrates about some equilibrium position based on a fixed frequency determined by the mass of the atom and the force of attraction or side of the bond between them. The pitch  $\nu$  of the vibration can be derived through the relation:

$$\nu = \frac{1}{2\pi} \sqrt{\frac{k}{\mu}}$$

Where:

- $\mu$  is the reduced mass of the two atoms which are vibrating, where  $\mu = \frac{m_1 m_2}{m_1 + m_2}$  and  $m_1, m_2$  are the masses of the atoms.

The low frequency behavior is that of the linear harmonic oscillator, which predicts that the frequency of vibration is independent of the amplitude of vibration, so the bond vibrates at a fixed frequency regardless of the amount that it is stretched or compressed. This models provide the basis for interpreting molecular vibrations, and it will enable to predict the vibrational frequencies that can be seen in the IR spectra. However, the vibrations of real molecules are imperfectly harmonic. When we talk about the harmonic model of the vibrations of a molecule, we have to also take into account that the molecules deviate from the harmonic form due to anharmonicity (something we will discuss here soon). For most vibrational



modes, the harmonic oscillator model is a reasonable approximation, particularly at small displacements around the equilibrium position.

### Force Constant and Zero-Point Energy

So when we talk about zero-point energy (ZPE), we are referring to the minimum amount of energy a molecule can have, even at 0 K temperatures. This is a quantum mechanical phenomenon coming from the fact that the particle (either an atom or a bunch of atoms) cannot have a completely rest state at its ground state. In quantum mechanics, the energy of a harmonic oscillator is quantized so that the molecule can only have certain energy levels. Then, the energy of these levels is:

Where:

- $E_v$  is the energy of the  $v$ -<sup>th</sup> vibrational level,
- $v$  is the vibrational quantum number (with  $v=0$  being the ground state),
- $h$  is Planck's constant,
- $\nu$  is the frequency of the vibration.

The background energy associated with zero-point energy is the energy for the ground state ( $v=0$ ):

$$E_0 = \frac{1}{2} h \nu$$

This implies that even at the lowest vibrational state of a molecule, it still has some energy left over. This energy is there to stay and is a pure consequence of quantum mechanical fluctuations present in every system with a nonzero temperature. One consequence of the existence of zero-point energy is that it has ramifications for the IR spectra of molecules. In a normal gas even with zero thermal excitation, molecules still occupy a

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finite amount of ground state energy, that translates in their vibrational modes and the IR absorption. The force constant  $k$  describes the stiffness of a bond between two atoms in a molecule, which in turn determines the vibrational frequency of the molecule. Higher force constant means a stronger bond and higher frequency vibration. The stronger the restoring force is, the larger the value of the force constant.

That is, the force constant of vibration is related to the frequency of vibration:

Where:

- $\nu$  is the frequency of the vibration,
- $k$  is the force constant,
- $\mu$  is the reduced mass.

In IR spectroscopy, the vibrational frequency of a molecule plays a major role in its absorbance properties. Generally, stronger bonds (higher force constants) vibrate at a higher frequency, corresponding to IR absorption peaks at higher wave numbers.

#### Harmonicity / Harmonic Potential / Harmonic Oscillator

The linear harmonic oscillator model describes molecular vibrations well, but assumes a restoring force exactly proportional to displacement. In reality, the vibrations of molecules behave differently from this idealized picture at larger displacements. This is what is called anharmonicity. In large vibrational amplitude, the potential energy curve is nonlinear and thus leads the harmonic approximation to fail. An alternative to the harmonic oscillator model is the Morse potential, which is a more accurate description of the potential energy curve that incorporates anharmonicity.

The math for the Morse potential is described by the equation:





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Based on the previous equations, we know that the potential is given by :

Where:

- $V(x)$ : Potential energy as a function of displacement  $x$ ,
- $D_e$  is the energy needed to break the bond (the dissociation energy),  $a$  is a constant that defines the potential well width,
- $x_e$  is the equilibrium bond length (the minimum of the potential position).

Due to the fact that the bond weakens further apart the restoring force does too, the Morse potential usually provides a much better representative of molecular vibrations. This creates a steeper line around the equilibrium bond length and a more horizontal line for larger displacements, which indicates that the bond is more strained as the atoms are pulled apart. More importantly, the Morse potential provides a more accurate representation of the vibrational energy levels. The harmonic oscillator model assumes that the energy levels are equally spaced, while the Morse potential predicts that the spacing between the energy levels decreases as the vibrational quantum number increases. This is because the molecule becomes less stiff when the bond is stretched, which lowers the vibrational frequency. The vibration levels in a Morse potential diagram are not equidistant. Unlike the harmonic oscillator model where the energy levels are equally spaced, the energy levels become increasingly closer together as the vibration progresses to higher quantum states. This anharmonicity leads to an observed IR vibrational wavelength shift, especially for large vibrational displacements or low frequency bonds. In reality, molecular vibrations are anharmonic, and the IR absorption spectra for a molecule will not consist of perfectly symmetric peaks. Instead, the peaks of the absorption bands may show asymmetry and shifts in position, offering further information about the molecular structure itself, bond strengths, and the environment of the molecule. This makes the Morse

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potential especially useful for explaining the overtones and combination bands commonly seen in IR spectra. Absorption peaks at frequencies higher than those predicted by the harmonic model result from the absorption of more than one photon at a time, or from more than one vibrational transition at the same time.

### 3.1 Vibration-Rotational Spectroscopy

We also consider several applications which combine the infrared and microwave regions in vibration-rotational spectroscopy. This is due to molecular energy states that are determined by vibration and rotation of the molecule. The two motions are typically coupled, and their net impact on the energy levels is observable in the infrared part of the electromagnetic spectrum. Unlike many other techniques, which enhance only specific molecular arrangements, vibration-rotational spectra convey information about molecular structure, bond lengths, and elemental masses. The energy levels for this type of molecule are allowed and play a major role in the analytics of this type of molecule as the energy carrying units in this type of molecule are both vibrational and rotational in nature. This will include understanding the basic principles of rotational and vibrational energy levels, the coupling of these motions, and the interpretation of the spectra in terms of more useful structure data characterizations.

Branches of Spectra: P, Q, and R

The absorption spectrum in vibration-rotational spectroscopy entails of a chain of absorption lines, that Transitions between the energy levels of molecules are the basic block of the understanding of molecular spectroscopy. (Transitions happen when a molecule absorbs or emits energy associated with the difference in energy between two quantum states.) Usually, these transitions fall into one of three categories, known as the P branch, Q branch, and R branch. The branches account for various kinds of transitions in the rotational-vibrational energy levels of a molecule. They are, in fact, closely connected to the changes in the rotational quantum number, which occurs during a vibrational transition



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as we have discussed previously in context of the P, Q and R branches. These branches represent vital sections of molecular diagrams critical for understanding molecular behavior and energy interactions, at the crux of molecular spectral analysis.

#### **P Branch**

Transitions in which one takes a single step down in rotational quantum number are called the P branch. That is to say, the transition goes from a higher rotational state (labeled by the quantum number  $J$ ) to a lower one (labeled by  $J-1$ ). From a molecular energy level perspective, this is an absorption process in which an energy transition occurs from a higher rotational level to a lower one, and therefore there is also a transition in the vibrational state of the molecule. The P part is normally seen at lower frequencies or low wave number in the spectrum.

**Vibrational-rotational spectrum** In a vibrational-rotational spectrum, transits will usually be represented as a series of lines. The P branch lines are located at lower wave numbers than the R branch. For the P branch, the corresponding energy differences between rotational levels can be shown. With successive values of rotational quantum number, energy levels approach closer. Thus the energy difference between adjacent rotational levels decreases. This means that the energy separation between the P branch adjacent lines actually decreases as the rotational quantum number increases, resulting in a narrower spacing because going from high-energy end to low-energy end of the P branch. This behavior reflects the fact that for rotational energy levels, increasing quantum number gives rise to ever closer energy levels. For molecular systems, this is an important phenomenon which makes it possible for determining the rotational constants and other similar properties of the molecule. The rotational structure of the molecule has particular value in the P branch, and you can use equations to derive structures such as moment of inertia from this branch. As a last point regarding the application of Lucy and Frankie, the appearance and distension of the lines in the P branch provides information about the physical nature of the molecule being analyzed. Finally, the P branch may have characteristic appearance of closely spaced lines,

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whose positions depend on the isotopic composition of the molecule, changing the distribution of mass segments and the respective rotational constants.

**Q Branch**

The Q branch is more similar to the P and R branches when transitions do not change the rotational quantum number. These are the transitions where the rotational state of the molecule does not change in the course of a vibrational transition ( $J' \rightarrow J$ ). Unlike in rotational transitions, in vibrational transitions the absorbed or emitted energy reflects just the change in the vibrational state of the molecule. Thus, the Q branch generally is manifested as a single, narrow line within the vibrational-rotational spectrum. This result reflects the fact that the line position is generally very close to the zero-point of the vibrational mode; the transition involves no change in rotational energy. Many molecular spectra lack a Q branch or show it only as a weak line because selection rules for rotational transitions favor transitions where the rotational quantum number changes (the P and R branches). On the other hand, according to the selection rules a change in the rotational quantum number is usually needed for a transition. Hence, both the P and R branches represent transitions that involve considerable shifts in the rotational and vibrational states, while the Q branch represents the transition that is purely vibrational without any change in the rotational symmetries. A defining characteristic of the Q branch is of its location along the entire spectrum. As there is no change in the rotational quantum number, the Q branch frequently appears as a single peak in the spectrum and can sometimes overlap with the zero point of the vibrational transition. The intensity of the Q branch is generally lower than that of the P and R branches due to the fewer transitions which fulfill the selection rules for such transitions. The Q branch can, though, be informative in regimes where the vibrational transition is much stronger than the rotation from continuum states or in symmetric species, allowing for transitions with no change in the rotational quantum number.

**R Branch**



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R branch: transitions for which the rotational quantum number increases by one. During these transitions, the molecule obtains energy in both vibrational and rotation modes, thus, the shift from lower rotation state ( $J$ ) to upper rotation state ( $J+1$ ). Consequently R branch is usually seen at the high frequency or higher wavenumber than P branch. This increase in frequency reflects the energy carried by the increase in the rotational quantum number. Inequity, the rotational lines within the R branch additionally form a sure pattern—this can be seen in the spacing of the trace in Fig. a. The pattern occurs because the energy accumulations of rotational lines become increasingly crowded with each upward change in the quantum number, thus the difference between the energies of higher rotational levels is diminished. So the separation between the lines in the R branch is reduced upon increasing the rotational quantum number. R branch transitions are basically transitions represented on the high energy side of the transitions spectrum with each transition corresponding to an increase in the rotational quantum number by one unit and a change in the vibrational state. The R branch, much like the P branch, contains important information regarding the rotational structure of the molecule. They give a lot of information about the rotational constants and the moments of inertia of the molecule through the spacing and the pattern of the lines in the R branch. In contrast, the R branch is often more vivid than the Q branch because the molecule has a high dipole moment. Moreover, the R branch is an important aspect of the molecular spectrum during transitions when the molecule is entering the higher vibrational states and the rotational and vibrational states are highly coupled.

#### **Born-Oppenheimer Approximation**

The Born-Oppenheimer approximation is a common assumption in quantum chemistry that simplifies the treatment of molecular systems by assuming that the motion of nuclei and the electrons in a molecule can be considered separately. The approximation assumes that the nuclear motion (nuclei are much heavier than electrons) could be treated in a way separate from electron motion. This means that the electronic wave function can be solved completely separately from the nuclear motion, treating the electronic energy as a potential energy that

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electrons usually react much faster than the nuclei. However, this approximation can break down if atoms in a molecule have large differences in atomic mass. And in particular, the case when molecules contain light atoms (hydrogen's) and much heavier ones (chlorine, iodine and other heavier elements) the motion of the nuclei can no longer be neglected. Hydrogen atoms, for instance, are relatively light, so they can wiggle quite well in particular vibrational modes. As the hydrogen nuclei motion becomes significant compared to the electron motion, one can expect deviations from the normal vibrational energy levels. For some molecules, the nuclei move enough to induce changes in the potential energy surface of the molecule, which then must be treated by coupling nuclear and electronic motion in a more involved way. For example, in molecules with heavier atoms bonded to hydrogen, such as hydrogen halides (HCl, HF, etc.), the large contrast in atomic masses between the hydrogen nucleus and the halogen can yield strong nuclear motion, especially in vibrational modes. Such nuclear motion allows for breakdown of the Born–Oppenheimer approximation, and non-adiabatic effects must be used to describe the system accurately. In such cases the electron wave function may no longer be able to respond instantaneously to the motion of the nuclei as is assumed in the Born–Oppenheimer approximation. This means that for cases in which the mass difference between the atoms is large, the approximation breaks down, and more advanced models that take into account the coupling of nuclear and electronic motions are needed.

### Large Nuclear Displacements

The Born–Oppenheimer approximation also fails in systems of widely displaced nuclei, namely systems with large amplitude vibrations. This approximation relies on the fact that nuclei vibrate around their equilibrium positions with small amplitudes, displacing the nuclei from their equilibrium positions small enough to not perturb the electronic wave function away from adiabatic separation from the nuclear motion. In certain cases, however, the displacement is large enough that the wave function, which reflects the distribution of charge about the nuclei, begins to depend on the position of the nuclei, nuclear motion. The potential energy surface of the molecule may change considerably when the displacements



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of the nuclei become large enough. As a result the electronic levels can intermingle, and the electronic wave function can no longer be considered a mere trial function of the nuclear positions. In these situations, the nuclear motion can no longer be separated from the electronic motion, and as a result, non-adiabatic effects need to be incorporated. This leads to complication of the nuclear and electronic motions, which can give rise to signs of non-adiabatic transitions between electronic states. In more practical terms, this can be seen in the case of molecules with large vibrational motions, or in the excited states, where we can have significant displacements from equilibrium positions. Thus, especially in large amplitude vibrational modes in molecules (which is the case for large molecular clusters or large polyatomic molecules), the assumption of small nuclear displacements is lost. The electronic wave function may not be able to instantaneously rearrange itself to these large displacements, leading to the breaking of the Born-Oppenheimer approximation.

#### **An Electron-Nuclear Coupling Stronger than Typical**

The Born-Oppenheimer approximation becomes especially useful when the coupling between the nuclear and electronic motions is weak. However, this approximation may fail in some systems, particularly those which are highly excited states or have strongly interacted with electron-nuclear bodies. Some molecular systems including transition metal complexes, molecules having large dipole moments, or systems with strong spin-orbit coupling that show strong coupling between electronic and nuclear motions. This breaks the adiabatic separation in the Born-Oppenheimer approximation and makes non-adiabatic effects quite significant in the treatment of the system. For example, a major case where this breakdown happens are molecules with strong electron-nuclear interactions (like transition metal complexes). This sometimes results in cases where excited electronic states of these molecules lie close in energy such that nuclear motions couple strongly to them so their electronic wave function cannot be considered adiabatically separated from nuclear positions. In these systems, the electronic structure of a molecule can change rapidly with nuclear motion, and so the assumption that electrons adjust instantaneously to nuclear configuration changes breaks down.

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One well-known example of these systems is the transition metal complex, where the interactions of the d-electrons of the metal with the ligands involving the nucleus-clutch of the motions with strong coupling. By contrast, these types of systems are especially susceptible to non-adiabatic effects, as the electronic state energy levels can depend very sensitively on the nuclear positions. For examples in the case of molecules possessing large dipole moments or systems with spin-orbit coupling the electron-nuclear coupling is strong enough to lead to deviations from Born-Oppenheimer approximation. These phenomena can be attributed to the breakdown of the Born-Oppenheimer approximation in such cases, leading to modifications of the expected energy levels, which could become evident as shifts in vibrational frequencies, modified rotational constants, or even the involvement of non-adiabatic transitions between states of different electronic lifetimes. The other is due to systems that have conical intersections, which is the scenario where two or more electronic potential energy surfaces approach each other very closely, which is also a case where the Born-Oppenheimer approximation fails. Similar conical intersections exist also in excited states, and these are seen to facilitate non-adiabatic transitions that can result in strong coupling between electronic and nuclear motions. This interaction is especially prevalent in the fields of photochemistry and photo physics, where the dynamics of the excited state are important to molecular evolution.

This is noteworthy because one of the Han and Yang's findings is the preferred decomposition of the mass of the difference between the Born-Oppenheimer approximation, which also decouples electrons and nuclei in certain situations. If a molecule gets excited from one electronic level to another because of light absorbed, this does not mean that a molecule was excited by an electronic jump from its ground to an excited state. The assumption that the nuclei can be treated as fixed while the electrons respond instantaneously to the change in electronic state becomes invalid. In systems exhibiting ultrafast dynamics, processes that happen on femtosecond timescales, electronic and nuclear motion are no longer separated by orders of magnitude in energy. During such rapid processes, the electronic and nuclear motions can become coupled and push the system out of the Born-Oppenheimer approximation. This is mostly the case for systems that





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are engaged in photochemical reactions, in which energy contained in the excited electronic state can couple to nuclear motion on a timescale for the system to flip between electronic states. Such processes demand a more detailed description, involving the coupling between nuclear and electronic degrees of freedom, which the Born-Oppenheimer approximation cannot sufficiently account for. Non-Adiabatic Transitions; The transitions that a molecule can get between the electronic states which are near energy, it is said that non-adiabatic transitions take place. The Born-Oppenheimer approximation breaks down in these instances because the molecule cannot stay in a single electronic state while moving like that of nucleus. The molecule can, instead of just having the electrons moving, it can actually change electronic states as the nuclei move and that will change the vibrational and rotational spectra.

Those transitions where the electronic and vibrational motions are coupled, i.e. not satisfying the parameters of Born-Oppenheimer approximation, but still possible are commonly referred to as vibronic transitions and they represent one of the most known fashions of breakdown of Born-Oppenheimer approximation. The transition is called vibronic when the molecule undergoes a simultaneous application of an electronic and vibrational transition. In these situations, the motion of the nuclei affects the electronic state of the molecule and the reverse holds true as well, resulting in a more coupled interaction than predicted by the Born-Oppenheimer approximation. The failure of the Born-Oppenheimer approximation manifests in phenomena such as avoided crossings (often seen as the splitting of levels that did not split in the simplified model), rotational anomalies and the emergence of additional vibrational modes not predicted by the simplified model. This would give further information of the coupling between the electronic and nuclear motions, giving deeper insight into the electronic structure and dynamics of the molecule. This means that the Born-Oppenheimer approximation is no longer valid, and this breakdown has important consequences in terms of the properties of the vibration-rotational spectra. There may be irregularities in the spacing of the P, Q, and R branches for example, or their respective intensities might be affected. The effects make it necessary to understand subtle details of the dynamics of the

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molecule and may also need sophisticated theoretical treatment to calculate the spectra.

**3.2 Vibrations of Polyatomic Molecules**

Vibrational spectroscopy is of fundamental importance for molecular structure and dynamic properties, particularly for polyatomic molecules, which are molecules that contain more than two atoms. In polyatomic molecules, vibrational modes become more complicated because they contain more degrees of freedom and thus more vibrational modes, each with their own frequencies. Chemists and spectroscopists can derive information about molecular geometry, bonding, and atom-atom interactions through the study of the vibrational modes of these molecules. When the bond between two molecules stretches and bends, they produce multiple frequencies that characterize the polyatomic molecules. These modes are affected by molecular mass, bond strength, and the symmetry of the molecule. Vibrational spectra thus obtained contain abundant information on molecular structure and dynamics. Here we discuss the key points of polyatomic molecular vibrations which will include selection rules for vibrations, normal modes of vibration, group frequencies and overtones, hot bands and Fermi resonance. All of these facets are crucial to understand the meaning attributions of vibrational spectra and provide a comprehensible apprehension of molecular vibrations.

**Vibration Selection Rules**

Selection rules in molecular spectroscopy determine the allowed transitions between different vibrational energy states. These criteria follow from the symmetry of the molecule and the way in which the molecule interacts with electromagnetic radiation. Selection rules for polyatomic molecules are more complicated compared with how they are for diatomic molecules as there are more vibrational modes and more complicated interactions possible. One of the main guidelines for a molecule undergoing a vibrational transition is that there must be a change in the dipole moment of the molecule. This is because the molecular state must couple to the electric field of the infrared radiation for



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absorption to take place. Consequently, for a transition to be infrared active, there must be a change of dipole moment through the vibration.

A vibration to be infrared active also has a selection rule, as follows:

$$\Delta q \neq 0$$

where  $q$  is the dipole moment and  $\Delta q$  is the change in the dipole moment once the vibrational motion happens. However, if a vibration does not cause a change in the dipole moment, it will not absorb infrared radiation and will be termed IR inactive.

Besides the dipole moment, the symmetry of the molecule also plays an important role in determining the activity of vibrational modes. Higher symmetry molecules also may have fewer IR-active vibrational modes since certain vibrational motion do not produce a change in the dipole moment or are symmetry-forbidden. For example, the IR inactive symmetric stretching modes of tetrahedral molecules, such as methane ( $\text{CH}_4$ ), do not result in a change in dipole moment for the molecule, as all hydrogen atoms move synchronously. Conversely, asymmetric stretching modes that result in a change in dipole moment, lead to IR activity. When discussing molecular vibrations, the Raman selection rules are also crucial. Raman scattering is the interaction of light with polarizability of the molecule. Firstly, the transition for Raman active modes must be due to change in polarizability of the molecule, according to selection rules. Some vibrational modes can be active in IR but inactive in Raman and the other way around.

### Normal Modes of Vibration

Normal modes of vibration (other than those due to free molecules) are key concepts in vibrational spectroscopy, especially in the case of polyatomic molecules. A normal mode is an individual vibration of the whole molecule, where all atoms move with the same frequency in a correlated way. Put differently, the entire atoms move together in some normal mode at once, in such a way that they don't disturb each other's motion. If we have a polyatomic molecule, the number of

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normal modes is related to the number of atoms of the molecule. Normal modes of vibration (excluding translational and rotation modes) For a molecule consisting of  $N$  atoms, the number of normal modes of vibration is  $3N - 6$ . This formula applies only to nonlinear molecules. The number of normal modes for a linear molecule is  $3N - 5$ , since one translational and two rotational degrees of freedom are excluded. The vibrational motion of molecules is of great relevance for their structure and properties, and is a cornerstone for techniques such as infrared (IR), and Raman spectroscopy. The motion of the atoms is relative to each other and can, therefore, consist of different patterns or “normal modes” of liquid molecule vibration. Every normal mode describes a particular type of vibrational motion, which can be excited by absorption of electromagnetic radiation, usually in the infrared range. Normal modes of vibration fall into two broad classes based on the direction of atomic movement (stretching modes and bending modes). This data is reliable not only for reflecting the fundamental interactions in the molecule but also for extracting valuable data related to the structure and dynamics of the molecule.

### Unit- 8 Stretching Modes

These are referred to as stretching modes, as can be seen from the slight alteration to the positions of the atoms along the bond axis of a molecule. The bond length toggles between compressed states and extended states as the atoms are pulled close and pushed away from one another in these modes. Pulsatory patterns are fundamental to comprehend how the bond strength and the bond length between two atoms affect those molecules physical properties and their reactivity. Stretching modes are divided into two sub-subcategories (that is, they're a subcategory of a subcategory) symmetric stretching and asymmetric stretching.

#### **Symmetric Stretching**

Symmetric stretching is a bond stretching mode that does not vary in width with respect to the center of a molecule. In this mode, all the atoms involved in the stretching motion, move in the same direction simultaneously, and interatomic distances are symmetrically displaced. Symmetric stretching is common in molecules where multiple identical bonds exist, such as in diatomic molecules, or



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in molecules with symmetric arrangements of atoms. In this mode, the bonds are expanding and contracting evenly, resulting in an even distribution of energy through the molecule. As an example, consider a simple linear molecule such as carbon dioxide ( $\text{CO}_2$ ); due to symmetric stretching, both  $\text{C}=\text{O}$  bonds are extended together, thus making the molecule longer or shorter along the bond axis. This symmetric stretching mode is generally lower-frequency than other vibrational modes, as the change in bond length is distributed over the entire molecule. Symmetric stretching modes are usually associated with the most intense peaks in infrared absorption spectra resulting from the high activity of the corresponding electric dipole moment change throughout the vibration. Molecules that exhibit so are undergo symmetric stretching where they exist in a more symmetrical state like  $\text{O}_2$  or  $\text{N}_2$ . The symmetric stretching modes are the simplest to find and describe in spectroscopic studies. Symmetric stretching modes, such as  $\nu_1$ , indicate infinitesimal shifts in molecular bonds that may contribute to the strength and nature of interactions in the molecular species. The strength of these vibrational modes can also provide insight into the polarizability of the molecule, as well as the molecular architecture.

#### Asymmetric Stretching

This mode is called asymmetric stretching where the atoms are moving in opposite direction causing an uncoordinated change in the bond length. For each bond length, one length rises while the other falls, and the atoms pull apart and go toward each other out of sync with each other, like a slowly spiraling lotus. Asymmetric Stretching is especially significant in molecules which have multiple bonds, whereas polyatomic molecules vibrate such different atoms do not shift in and out to the same extent. In a species such as carbon dioxide ( $\text{CO}_2$ ), asymmetric stretching refers to the lengthening of one of the  $\text{C}=\text{O}$  bonds while the other bond shorten and the central carbon remains roughly stationary. Asymmetric stretching, the atoms change its movement along the bonding axis is different, so it does more complex motion than symmetric stretching. Again, this mode is characterized by higher frequencies than symmetric stretching, because this involves a much greater change in the bond length. An example of asymmetric stretching can be seen in

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molecules consisting of dissimilar atoms bonded with covalent bonds, where bond length is altered unequally, such H, O or CH<sub>3</sub> OH. Asymmetric stretching in molecular spectroscopy is significant as it reflects the differential forces acting on the atoms, thus providing insight into the different interactions. Asymmetric stretch modes are usually found in the higher energy states for vibrational analysis as the vibrational motion in this type is more complex. Since these modes are very active and contribute significantly to the vibrational absorption of molecules, they manifest as large peaks in infrared absorption spectra. Paralleling asymmetric stretching is an important mode in the research of polyatomic molecules and the information it contains regarding the molecular structure and couplings of the various bonds.

**Bending Modes**

In contrast, bending modes are vibrational motions in which the angle between two bonds is changing, as opposed to the bond length itself. (9) These modes refer to a variation in the bond angle rather than the bond length, resulting in a different kind of atomic displacement. In bending motions, atoms can also be displaced in a molecule plane (in-plane bending), or can be moved toward or away the plane (out-of-plane bending) and numerous bending subtypes can be defined according to the atoms direction of the displacement. The modifications in geometries become vital because of their impact on most of the physical properties or reactivity of the molecules.

**In-plane Bending (Scissoring)**

In-plane bending (Scissoring); The angle between two bonds of the same plane of the molecule over changes. In this mode, the atoms belted by the bonds slide toward and away from each other, but stay in the plane of the molecule. The resulting motion is reminiscent of the scissoring motion, with the bonds “opening” or “closing,” in synchronicity. Modes related to in-plane bending are changes in bond angles in the plane of the molecule and are seen most often in non-linear or bent molecules.



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An example of in-plane bending mode is that of water ( $\text{H}_2\text{O}$ ), in which the two hydrogen atoms move towards each other or away from each other, changing the angle between two O-H bonds. Movement in water occurs via bending, a process during which the bond angle between the oxygen and hydrogen atoms changes, which in turn affects the molecular geometry and, in turn, the physical properties of the molecules, the part of the molecule's dipole moment and intermolecular interactions. The frequency of the in-plane bending motion is usually in the infrared region of the spectrum, and this mode is often intense in the vibrational spectra of bent molecules. Scissoring modes are particularly important in angular or bent molecules as they inform on the variation of the bond angles in the molecule. In general, the bending modes in plane have higher frequency compared to the stretching modes, since bending motions usually need more energy to enable because of bond angle distortions.

#### **Bending (Rocking, Wagging, Twisting) Out-of-plane**

Out-of-plane bending refers to changes in bond angles in directions orthogonal to the molecule plane. These bending motions represent movements such as rocking, wagging, or twisting, where the atoms participating in the bond move in a direction perpendicular to the plane of the given molecule. Out-of-plane bending modes can also be more detailed than in-plane bending modes and the types of out-of-plane bending modes will be determined by the molecular geometry and relative displacement of the moving atoms.

The out-of-plane bending modes can be classified into the following types:

- **Rocking:** In rocking, the bonds move in opposite directions, and the atoms “rock” back and forth around an axis. Such motion is seen most often in linear or almost linear molecules, where the atoms rotate around a central atom in such a way that the bond angles are perturbed.
- **Wagging:** Wagging is a bending motion in which atoms move in and out of the molecular plane, similar to the motion of a wagging. This does occur more common for most asymmetrical atoms in which the atoms are shifted in opposite directions but only about a plane.

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- **Twist:** Twist is a way of rotation where atoms of a molecule rotate through an axis resulting in change in bond angle and causing non planar geometry of the molecule. Twisting modes often appear in large molecules with flexible structures that are capable of undergoing large change in orientation.

Molecules with more complicated structures, like polyatomic species that have multiple bonds in separate planes, tend to show out-of-plane bending motions. The bending modes are particularly critical in the analysis of large organic molecules and biological macromolecules since bending motions affect the global conformation of the molecule as in its interaction with other molecules. The frequency of the out-of-plane bending modes varies significantly based on molecular architecture and bending motions. For example, the twisting motion usually happens at lower frequencies than rocking or wagging motions. Out-of-plane bending modes may sometimes be more challenging to interpret, as they represent more complicated deformations of the molecular geometry; however, they still provide important information about molecular flexibility and the capacity for alteration and/or conformational change within a previously static molecule.

**Echo Harmonics, Frequency Groups**

But in molecular vibrational spectroscopy, group frequencies are the characteristic vibrational frequencies associated with specific functional groups within a molecule. These frequencies depend on the bonding context and the species of atoms involved. In fact, group frequencies are especially useful for detecting specific functional groups, since they are more consistent for a certain kind of bond.

For example:

- The C-H stretch of hydrocarbons usually shows up near  $2900\text{ cm}^{-1}$ .
- The carbonyl C=O stretch normally occurs in the  $1700\text{ cm}^{-1}$  region.





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- The O-H stretch from alcohols and phenols typically occurs in the range of about 3200-3600  $\text{cm}^{-1}$ .

These group frequencies are affected by the atom masses, strength of bonds, and any activity with adjacent atoms or groups. Polyatomic IR spectra contains overlapping bands due to various functional groups vibrations at individual group frequencies. Harmonics are higher frequencies of vibrations at integral multiples of the base vibrational frequency. These overtones can be seen in the IR spectrum at about two, three, or four times the frequency of the fundamental mode. Overtones, however, are usually very weak in intensity compared to the fundamental bands because the transition probability for overtone absorptions is orders of magnitude lower than for fundamental transitions. So, while overtones generally don't amount to much in most isolated molecules, they're still relevant in cases of large amplitude vibrations or highly excited vibrational states. Overtone bands not only provide information about the mechanics of the matrix element for the molecular vibration, but, through the intensity and position of a series of overtone bands, can also give an indication of the anharmonicity of the molecular vibration, as anharmonic potentials must lead to overtone bands.

#### Hot Bands and Fermi Resonance

Hot bands are vibrational transitions that take place when a molecule is in an excited vibrational state at temperatures above absolute zero. Generating vibrations through thermal energy at very high temperatures. Most of the molecules will be in excited states in terms of vibration at high temperatures. Unlike transition from ground state to excited state, these molecules transition from an excited state to a higher vibrational state when absorbing infrared radiation. These transitions are called "hot bands." The hot bands are usually seen at higher wave numbers as compared to the principle vibrational transitions. They are generally less intense than fundamental bands because fewer molecules occupy an excited vibrational state at a given temperature. But the intensity of hot bands is temperature dependent, since more molecules can occupy higher vibrational levels. Hot bands are indeed an important part of most IR spectra, whilst providing key insights into the temperature dependent distributions of molecules in each vibrational state; as such,

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they can also be used to study the thermodynamics of molecular vibrations. Fermi resonance occurs when two of such vibrational modes of similar frequency are involved, which causes an alteration of the frequencies of both modes. This interaction causes the two vibrations to couple, producing a phenomenon where the two modes affect each other and their energy levels are mixed. Fermi resonance commonly takes place when the energy separation between the two modes is small ( $200\text{ cm}^{-1}$ ) and can have the effect of splitting or shifting bands in the vibrational spectrum.

**3.3 Metal-Ligand Vibrations**

Metal-Ligand Vibrations the vibrational modes of a metal complex where a metal ion is coordinated to one or more legends. The information regarding the bonding, structure, and geometry of the metal complex is available from these vibrations, which can be studied by infrared (IR) spectroscopy. In particular, this vibration can be studied by examining the frequencies of the metal-ligand bonds, which shows how different metal-ligand interactions the metal and legends have. Vibrational modes typically fall into the far infrared (Far IR) region of the spectrum, but other regions can be observed in accordance with the type of bond. High-frequencies for metal-ligand vibrations enlighten the nature of the metal-ligand bond, its strength, the floridity of the complex, and eventually even with the electronic structure of the metal center. Normal coordinate analysis and other sophisticated methods in handling these vibrations are prominently studied and employed in polyatomic molecules. Together this can help chemists determine the identity of the complex, based on the presence of specific functional groups (fingerprints) and/or overall group frequency.

**Far IR Region Spectroscopy**

Typically defined as wavelengths between 15 to 1000 micrometers (or frequencies between  $30\text{--}200\text{ cm}^{-1}$ ), the Far IR Region of the electromagnetic spectrum is crucial for the study of low-energy vibrations, including metal-ligand complexes. The connections being referred to in-between the metal-ligand bond is responsible for the stretching and bending modes of these two electrons, as seen in the Far IR area. In contrast to the mid-IR region, where standard organic molecule vibrational



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modes are observed, the Far IR region is defined by reduced energy. Since heavier elements, such as metals, have much lower frequencies for metal-ligand vibrations than lighter elements, this region is very relevant for getting insight into the vibrational profiles of heavier atoms. For instance, the stretching frequencies of metal-ligand bonds typically fall in this region, which is well-suited for discerning the bonding environment surrounding a metal center. For complexes of this type, the Far IR spectra can even give information on the metal-ligand bond force constants. The stiffer the bond (larger force constant) the higher the frequency of the vibration. In contrast, weaker bonds vibrate at lower frequencies. As an example, stretching vibrations of metal-oxygen (M-O) bonds in transition metal oxide complexes are usually seen in the Far IR region. Far IR spectroscopy essentially focuses on the identification of the metal-ligand stretching vibration appearing in the spectrum as rising peaks. The peaks can give insights about the coordination number, geometry, and type of metal ligand interaction. As an example, the metal-ligand vibrations in octahedral complexes will be different from those in tetrahedral complexes, thus gain information about the geometry of the metal center. In addition, metal-ligand vibrations may be affected by ligand field strength, the oxidation state of the metal, and type of legends. With the two SERS active vibrational frequencies lying in the fingerprint region, as well as the basic metal-ligand stretching, the Far IR region exhibits the bending modes of the legends themselves, particularly the bending of metal-ligand bonds. They usually fall at slightly lower frequencies than those for stretching vibrations and can be used to help determine the types of legends attached to the metal center. The Far IR (far-infrared) spectra of metal-ligand complexes are information-rich and can be employed to establish the presence of the required metal complex, determine the geometry of metal-ligand coordination and gain studies on transition through changing legends, oxidation states or other structural changes.

#### Normal Coordinate Analysis

Normal Coordinate Analysis is a Mathematical function which is used to study the vibrational modes of polyatomic molecules such as metal-ligand complexes. Normal coordinate analysis seeks a transformation of the coordinates where the vibrations

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of a molecule decouple into a set of independent vibrations called normal modes. Each normal mode is a specific vibration, like a bond stretching or bending motion, that has its own frequency. Normal coordinate analysis is fundamental for describing how the metal and ligand moves with respect to each other in a vibration in metal-ligand complexes. This approach sums the energies of the various normal modes to give the total vibrational energy of the system, providing a mathematical route to solve it based on quantum mechanical principles. The description of the system starts by the generation of the potential energy surface for a system, which describes the energy of a system as a function of atoms positions. From this PES, the system's force constants can be extracted. Force constants give valuable insight in the strength of the bonds and the stiffness of the metal-ligand interaction. The frequencies of the normal modes can be determined by solving the equations of motion for the system. Normal coordinate analysis makes possible the extraction of metal-ligand vibrational coupling. The metal and the ligands are not isolated from each other in a metal-ligand complex, rather, they interact through their vibrations. It describes how this coupling affects the vibrational frequencies that appear in the spectrum and allows further understanding of the nature of normal modes including how these higher order interactions affect or contribute to normal coordinates. The vibrational modes are coupled, for example in a metal-carbonyl complex (for transition metal carbonyls shown below), the metal-ligand vibrations (metal vibrations and CO ligands stretch are not independent. Normal coordinate analysis can also be performed on these coupled vibrations in order to provide insight into the bonding interactions of the metal with the ligand. Such analysis can also assist in identifying the relative contribution from each atom in the vibration, providing insightful information regarding the coordination environment in proximity to the metal center. In the context of the metal-ligand complex spectra, normal coordinate analysis can also be used to help in the assignment of overtones and combination bands. These bands occur when a molecule absorbs multiple quanta of energy, or makes several transitions at once. Let's do this for the frequency terms again, normal coordinate analysis makes it possible to characterize these intricate vibrations and how they contribute to the overall spectrum.



#### Understanding group frequencies using fingerprints

The concept of fingerprints and group frequency interpretation are the most important used in the interpretation of IR spectra including that of metal-ligand complexes. In IR spectroscopy, a fingerprint is a unique set of vibrational frequencies that can be used to identify a specific molecule or complex. The fingerprint regions can be located in the mid-IR region but also have the opportunity to exist as vibrational states within certain metal-ligand vibrations in the Far IR region. So the fingerprint region can be critical for determining what kind of metal-ligand bond we're looking at, since each complex will yield a fingerprints of vibrational frequencies for the given metal-ligand bond. The nature of the metal center, legends, geometry and oxidation state of the metal will dictate these frequencies. Researchers can identify the metal-ligand complex by comparing the observed frequencies to standard reference spectra. As an illustration, in the case of a metal-carbonyl complex, the stretching frequencies corresponding to M-C bonds in the Far IR region can serve as an identifying feature of the complex and can thus differentiate it from other compounds. Likewise, CO ligand bending vibrations can also be classified as a feature that offers identity in the fingerprint region, separating the complex from other metal-ligand species. Group frequency interpretation 215 involves the evaluation of modes consistent with certain functional groups or coordination environments. It has been reported that a bundle of metal-ligand complexes can give rise to vibrational frequencies characteristic of the metal-ligand bond formation. Group frequencies (see IR spectrum section, above) can be helpful for the identification of legends or specific bonding motifs in metal complexes. For instance for metal-ox we can see from stretching frequencies of the OA bonds that this bond have a stretch frequency in Far IR region. These were considered to be the group frequencies for the M-O bond which helped be in identifying ox legends in the complex. Typical stretching frequencies of a functional group in coordination complexes with phosphate or isocyanides legends can be similarly discovered in an IR spectrum and analyzed to gain more insight into the species surrounding the metal center. This grouping of frequencies is especially helpful while analyzing large-series of metal-ligand complexes, where one can observe trends and relations to the type of ligand and the vibrational

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properties observed. The legends are assigned according to group frequencies corresponding to respective metals.

**Unit -9 Instrumentation and FT-IR**

Fourier Transform Infrared Spectroscopy (FT-IR) is one of the most popular analytical techniques for sampling the vibrational modes of molecules and retrieving information about its molecular structure. FT-IR works on the premise that bonds in a molecule have distinct absorption frequencies in the infrared region of the electromagnetic spectrum, thus, it provides chemical information at the molecular level. FT-IR differs from classical dispersive infrared spectroscopy as instead of directly converting raw data into a readable spectrum, raw data is first collected and then transformed into a readable spectrum through the use of Fourier transformation. This groundbreaking approach led to a transformative improvement in infrared spectroscopy, offering enhanced specificity, resolution, and rapid acquisition of high-quality spectra with less effort.

**FT-IR: Principles of Fourier Transform Infrared Spectroscopy**

FT-IR spectroscopy is based on the basic principles of infrared absorption spectroscopy. Infrared radiation interacts with molecules in a way that causes their bonds to vibrate. Unique bonds in a molecule absorb infrared radiation at different fingerprints matching the vibrational frequencies of the specific bonds (C-H, N-H, O-H stretching etc.). The absorption spectrum obtained gives a molecular fingerprint of the sample analyzed. Unlike conventional IR spectroscopy, FT-IR collects data over a wide frequency range simultaneously. In conventional IR spectroscopy, the sample is illuminated with a monochromatic beam of infrared radiation, and the transmitted light is detected at various wave numbers or frequencies in order to generate a spectrum. This technique, called dispersive infrared spectroscopy, works by dispersing light into constituent wavelengths. FT-IR spectroscopy is based on a Michelson interferometer; this is essentially the key to its functioning, and it is different from the older technique. Their interferometer consists of two beams of light of



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different optical path lengths interfering with one another and the resulting pattern is subjected to a mathematical transform known as a Fourier transform to yield a spectrum. It had some advantages including but not limited to better signal-to-noise and resolution improvement and faster data acquisition. The FT-IR spectrometer generates a wide range of infrared light at multiple wave numbers. This light is focused at the sample, where it interacts with the sample and excites different bonds in the molecules to vibrate. Vibrational transitions occur when the frequency of the incident infrared light is equal to the same frequency of vibration of a particular bond in the sample. The light that comes out of the sample is then routed through the interferometer, generating an interference pattern.

The next component of the interferometer is typically two mirrors; one fixed and the other able to move. A mirror that can change its position adjusts the optical path length of one of the light beams. The optical path length difference is responsible for the interference pattern when the two beams combine back together. That interference pattern encodes information about the frequencies at which the sample absorbs infrared light. Then, the interference signal, called the interferogram, is acquired by the detector. The interferogram shows the data vs optical path difference rather than frequency. To derive the useful details of the sample's vibrational modes from the raw data in the form of an attenuated light beam, the interferogram needs to be mathematically transformed into a normal absorption spectrum by a calculation known as a Fourier transform. The signal we do this on is actually the interferogram that we have mentioned previously, and the process is known as using a Fourier transform in order to obtain the IR absorption spectrum in the frequency domain. From such a spectrum details of the intensity of absorption as a function of frequency (or wave number) are obtained, which are related to the vibrational modes of the molecule. This yields an intricate spectrum that shows absorption peaks that correspond to different molecular vibrations, enabling determination of functional groups and bonding

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information pertinent to the sample. FT-IR is superior to traditional IR methods because it collects an entire spectrum in a single scan instead of measuring each wavelength one at a time. This greatly accelerates the data collection process. Moreover, compared to dispersive IR instruments, the interferometric approach affords a higher sensitivity and resolution. There are a number of benefits of FT-IR due to the instrument itself, as well. The detectors for FT-IR instruments are piezoelectric or thermoelectric detectors and are very sensitive in order to detect low- concentration samples. Beyond just fewer moving parts helping with reliability, the simplicity of the instrumentation also translates to lower maintenance costs.

### Speed and Efficiency

One major advantage of FT-IR (Fourier Transform Infrared) relative to more traditional dispersive IR methods is the ease and speed at which spectra can be collected. In a traditional IR system, the spectrometer is required to step over various wavelengths one at a time. Scanning individual samples can take a long time; seconds to minutes for each spectrum to be collected. It typically employs a moving monochromatic or a diffraction grating to select single wavelengths of infrared light, thus it needs to scan over a wide spectral range. FT-IR spectroscopy, on the contrary, relies on a different technique. FT-IR works differently, instead of scanning a single wavelength at a time, FT-IR uses an interferometer to acquire all wavelengths at once in an interferogram. Following this, the interferogram is run through a Fourier transform algorithm to produce the spectrum. FT-IR spectrometers can then record the full spectrum within seconds or less. FT-IR is a much faster process than traditional methods since it collects data across a wide range of frequencies at the same time. Advantages of using the BioID for application high throughput In pursuit of high throughput in which many samples need to be analyzed at once, this speed is extremely beneficial. Moreover, it enhances the throughput





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in experiments and analytical pipelines, enabling scientists and technicians to generate more data in shorter periods of time.

#### High Sensitivity

FT-IR spectroscopy is very sensitive, which is one of its main benefits. Sensitivity in the context of a spectrometer is linked to the low concentration of the sample and the precise determination of infrared absorption. The sensitivity enhancement of FT-IR systems is primarily attributed to the higher signal-to-noise ratio, which is further improved through mathematical processing of the interferogram. For FT-IR spectrometers, it is common to average multiple scans to enhance the data quality. This common technique is called signal averaging, which will be used to reduce random noise in the spectrum and improve signal clarity. The outcome is a better description of the vibrational modes that the sample exhibits, even if the material being measured is present only at trace amounts. Consequently, FT-IR is well-suited to applications in which the detection of minimal volumes of substances is paramount. In the case of environmental monitoring, FT-IR can detect and quantify the pollutants at very low concentration. FT-IR is equally employed in the pharmaceutical sector, one addition is drug molecular characterization, and the other isotyping routes to identify for trace components in complex chemical mixtures.

#### Improved Resolution

**FT-IR Spectroscopy:** FT-IR spectroscopy offers higher resolution than traditional dispersive IR systems. In infrared spectroscopy, resolution is the separation between adjacent bands that still can be used to distinguish them in a spectrum. Higher resolution makes it possible to resolve individual vibrational modes of a molecule more clearly, thus informing our understanding of the sample's structure and behavior at the molecular level. In FT-IR the resolution depends both on the maximum optical path difference (OPD) in the interferometer and the number of data points

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collected. FT-IR instruments manipulate the interferogram mathematically and thus can extract higher-resolution spectral information. Higher precision and more detailed spectrum than when using more traditional methods based on scanning over the individual wavelengths are achieved with the Fourier transform algorithm; this allows the interferogram to be converted into spectrum. These two improvements are paramount for applications such as biological macromolecules, where accurate spectral features need to be resolved. FT-IR enables the unambiguous detection of individual bonds or functional groups in a sample, hence FT-IR is an indispensable technique for molecular characterization in chemistry, biology, and material science.

## No Scanning Monochromatic Required

The primary and perhaps, most definitive part of a traditional dispersive IR system is its scanning monochromatic which isolates individual wavelengths of light for detection. This necessitates mechanical moving parts, like mirrors or gratings, which can wear out over time and need regular maintenance. And also, these moving parts could lower the overall reliability and steadiness of the instrument. FT-IR spectroscopy, on the other hand, allows using an FTIR system, which does not require a scanning monochromatic. Instead, FT-IR uses an interferometer, which splits the incoming light into two beams and recombines them to form an interference pattern. This produces an interferogram that contains information on all the wavelengths of the infrared light in parallel. Therefore, the FT-IR system is able to output the sample spectrum directly from the Fourier transform algorithm in the time domain in which the interferogram is processed without mechanical scanning. All this means the absence of moving parts and improved stability and reliability of FT-IR spectrometers. FT-IR systems have lower maintenance costs and longer lifetimes due to fewer wear-and-tear mechanical parts. Additionally, eliminating the need for a monochromatic speed up data collection by avoiding time-intensive scans across the different wavelengths.



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### Quantitative Analysis

Qualitative analysis: FT-IR spectroscopy is not only more useful for qualitative analysis; it is highly efficient for quantitative analysis. FT-IR is employed in qualitative analysis to identify functional groups, chemical bonds, and molecular structures based on characteristic infrared absorption patterns. FT-IR can be used in quantitative analysis to measure the concentration of specific components within a mixture. Its high resolution combined with sensitivity is especially beneficial in quantification of substances, even in complicated mixtures such as biological samples. The intensity of absorption of a particular vibrational mode is proportional to the concentration of the associated component, and hence Beer's law is established; that is, the intensity of an absorption band can be used to quantify concentration of a particular compound. The repeated scans increase the reproducibility and precision of FT-IR measurements which is one of the advantages for quantitative analysis. FT-IR instruments offer high-quality spectra with minimal noise, allowing for accurate and reliable data in the industries like pharmaceuticals, food and beverage, and environmental monitoring. FT-IR is often used in the pharmaceutical industry to monitor the required amounts of active ingredients in drugs.

### No Sample Preparation

FT-IR spectroscopy also has another big advantage it usually needs very little sample preparation. In many cases, samples can be analyzed without the need for tedious preparation techniques or complex modification of the samples themselves. FT-IR can study solid samples whether as a powder, thin films, or even bulk directly, while liquid samples can often be studied directly with no elaborate special treatments. In certain situations, special accessories like attenuated total reflectance (ATR) or transmission cells are utilized to optimize the quality of the measurement; however, these accessories are often straightforward to use and do not need intensive sample pre-treatment. For instance, ATR-FT-IR enables the

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probe of the sample directly on crystal faces, sometimes without any dilution, complex treatment, etc. The ability to analyze samples without elaborate preparation not only saves time, but also minimizes the potential for contamination or degradation of the sample. It also removes the necessity of dangerous reagents or solvents which may be needed in other types of assay. This is especially useful when studying materials that are reactive to their environment like biological samples, or for studying particularly complex mixtures. FT-IR Applications-IR spectroscopy is an all-rounder and can be applied for the analysis of various sample types. While FT-IR can analyze a sample regardless of its physical state (gaseous, liquid or solid), the method is extremely versatile and has a wide range of applications in both research and industry. A few examples of applications of FT-IR are listed below:

- **Polymer Characterization:** FT-IR is utilized for identifying and characterizing polymers, monitoring polymerization reactions, and finding contaminants in polymer samples.
- **Biological and Medical Applications:** FT-IR is being gaining the popularity for the analysis of biological tissues, cells and fluids. It assists in determining specific functional groups present in different biomolecules, making it advantageous for drug development and diagnostics.
- **Environmental Monitoring:** FT-IR is utilized in the detection of pollutants in both air and water, as well as for monitoring of greenhouse gases, volatile organic compounds (VOCs), and particulate matter.
- **Materials:** FT-IR is commonly used in the study of materials, for example, coatings, paints, and thin films.



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FT-IR enables the identification of many different materials and is therefore incredibly useful, as it can be applied to opalescent samples (many composite substances).

#### Improved Accuracy, Precision and Reproducibility

A critical aspect of FT-IR spectroscopy is its highly reproducible results, a fact that is a prerequisite in various industries that depend upon routine analysis and quality control. High sensitivity combined with low sample preparation and the ability to average multiple scans leads to spectra that are accurate and reproducible. More frequent in quality control environments like pharma and chemicals industries, consistency and reliability of data is of utmost importance. FTIR allows the identification of compounds to be done in a repeatable manner and ensuring the specifications of products and preventing contamination. The high reproducibility of FT-IR measurements is a factor that minimizes the error possibilities as well as assures that the data collected over a period of time are consistent and serves long-term monitoring and routine testing approaches excellently.

#### FT-IR Spectroscopy Uses

The introduction of FT-IR (Fourier-transform infrared) spectroscopy changed the landscape of analytical chemistry due to its fast, nondestructive, and sensitive capabilities in the identification and characterization of chemical substances. FT-IR spectrometers are employed in a variety of industries and areas of research, such as environmental analysis, material science, foodstuffs, environmental science, chemical analysis, pharmaceuticals, forensic science, etc. Tapping into the molecular vibrations of different bonds, this method provides rich information on molecular structure, chemical makeup, and functional groups. FT-IR spectroscopy has some of the main applications given below:

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## Analysis and Identification of Chemicals

FT-IR Spectroscopy is a potent analytical method for the quantification and identification of chemicals in the target of analyze samples. The methodology used is capable of identifying organic and inorganic functional groups and bonding types. FT-IR imaging creates unique molecular fingerprints in the infrared spectrum, which can be used for the identification of unknown compounds by comparing them to known spectra. FT-IR is a common technique used in chemical research to characterize the molecular structure of compounds. As an instrumental technique, The FT-IR spectrum is known to give information about the molecular environment and interactions between functional groups through the position and intensity of the absorption bands. FT-IR is also used in industrial applications when it comes for quality control and in terms of raw materials as well as finished product identification. It is also employed as an analytical technique to assess compound purity as well as verify chemical composition and identify potential contaminants. The non-destructive nature of FT-IR and the associated lack of complex sample preparation make FT-IR an excellent technology for routine quality assurance in production.

## Environmental Monitoring

Environmental monitoring using FT-IR Spectroscopy; FTIR Spectroscopy is an important tool for environmental monitoring, which involves the analysis of air, water, and soil samples for pollutants. It is capable of sensing various environmental pollutants including volatile organic compounds (VOCs), greenhouse gases ( $\text{CO}_2$ ,  $\text{CH}_4$ ), industrial effluent, and particulate matter. The sensitivity and ability of FT-IR to rapidly analyze complex samples make the technique a mainstay of environmental monitoring, particularly outdoors. FT-IR spectrometers are commonly used in air quality monitoring, enabling the detection of trace gases and pollutants. FT-IR is used in the control of the water quality for the detection of contaminants, such as pesticides, herbicides,



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and heavy metals. Soil for organic compounds, pollutants, and other substances that may affect soil health FT-IR spectroscopy is also employed in atmospheric studies, whereas it is used to monitor the air composition and trace gases leading to climate changes such as the human-made greenhouse gases. FT-IR, on the other hand, enables real-time, in-situ measurements which provide a better understanding of environmental pollution and contribute to mitigation efforts.

#### Applications in the Pharmaceutical and Biomedical Industries

FT-IR spectroscopy is commonly used in pharmaceutical and biomedical industries. One important application is to confirm raw materials, intermediates, and finished products purity and quality. FT-IR is used by pharmaceutical companies to verify that drug APIs comply with specific purity requirements. To investigate recipients (inactive ingredients) and discover possible impurities in pharmaceutical formulation, FTIR can also be employed. FT-IR is instrumental in investigating drug-receptor interactions, elucidating bimolecular structures and structure-based drug design in drug development. FT-IR can show how a drug binds to a receptor site or interacts with other biomolecular, which can help to understand its mechanism of action. In addition, FT-IR spectroscopy is also used for biological samples, including tissues, cells, and fluids. It is utilized in research to investigate variations in the chemical configuration of biological components as a function of disease evolution, aging or therapy. FT-IR is non-invasive with respect to biological samples, providing opportunities to study live cells or tissues without compromising their integrity, thus rendering FT-IR an important tool in medical sciences and diagnostics. FT-IR is also used in counterfeit drug identification for drug safety and protection of the public health. It has been found that counterfeit drugs also contain impurities or differences in composition, which can be detected in FT-IR spectroscopy, making it a reliable quality control method for the pharmaceutical industry.



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### Polymer and Materials Science

FT-IR is widely applied in materials science field such as polymers and materials characterization, chemical composition, and molecular structure. FT-IR can identify functional groups and bonding configurations in long chains of repeating units, which make up polymers. This makes FT-IR, therefore, absolutely indispensable in polymer synthesis, analysis and characterization. Polymer degradation; One of the leading applications of FT-IR in polymer science. “Polymers can degrade if they’re exposed to heat, light, oxygen, or when they undergo chemical reactions. Through the chemical structural alterations, researchers and manufacturers assess the stability of materials and their longevity, which can be detected using FT-IR. FT-IR is also useful for investigating curing processes in thermosetting polymers. It allows to monitor the progress of polymerization, thus giving an insight of cross linking and creation of new bonds. FT-IR also provides information on additives and impurities in polymeric materials, such as stabilizers, plasticizers, and fillers that may influence the performance of the material. FT-IR can also be used in the study of new materials such as nonmaterial’s and other coatings. The combination of these techniques allows researchers to be specific in making materials with tailored properties such as better strength, flexibility, or environmental resistance, FT-IR analysis.

### Food and Agriculture

Many applications have been developed to use FT-IR spectroscopy in the food industry. It is employed to study the makeup of food products, track quality, and enforce safety regulations. Moisture, fat, and protein are important factors that will affect the nutritional value and quality of food products. FT-IR is widely used for the determination of food moisture, fat content, and protein content. The technique can also help detect additives, preservatives, or contaminants in food, making it an efficient





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tool for monitoring food safety. For instance, it can be used to identify the presence of pesticides, antibiotics, and other chemical residues in food products, thus verifying whether they are safe for human consumption. In agricultural research, FT-IR spectroscopy is applied for plant sample analysis. It can also assess how nutritious the crops will be, monitor the health of the plants and identify any diseases or pests presence. A similar use of FT-IR is in the examination of soil constituents, including the detection of contaminants or nutrient deficiencies in the agricultural field.

#### Forensic Science

FT-IR spectroscopy is also a useful analytical technique in forensic science for examination of trace evidence that may lead to the evidence on the case in criminal investigation. FT-IR is known for its role in forensic analysis of society wherein forensic scientists identify substances present at crime scenes such as fibers, paints, drugs, explosives, and materials. This is especially important doing fibers and paints, as FT-IR shows what chemicals are in the fibers they will identify what kind of molecules. This is especially important in instances where fibers or paint chips located at a crime scene may connect a suspect to the crime. For drug analysis, FT- IR can be used to detect the presence of illegal drugs, including narcotics or synthetic drugs. FT-IR is uniquely non-destructive, enabling forensic scientists to examine the nature of the substance before it has been manipulated (The non-destructive nature of FT-IR, 2023). This is particularly important for analyzing explosives and gunpowder residues, which can be used in terrorist attacks or violent criminal activities.

#### Materials Characterization

FT-IR spectroscopy has been used extensively in materials characterization, such as thin films, coatings, and composite materials. The lab will use a powerful new technique to help tell them the detailed composition and molecular structure of materials — a key tool for anyone developing new materials with certain properties. In FT-IR analysis of

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thin films and coatings, the molecular structure, thickness, and uniformity can be analyzed. These insights are important for the design of high-efficiency coatings for electronics, optics and other applications. Composite materials with components from polymeric elastomers, polyethylene, etc. have been studied as well using FT-IR spectrometry. This way, FT-IR allows researchers to improve the performance of composite materials by determining the chemical composition of each component. Moreover, FT-IR is applicable in corrosion and surface treatment analysis, as it enables the investigation of the chemical changes occurring on the material surface over time.

**Multiple-Choice Questions (MCQs)**

**1. In infrared spectroscopy, molecular vibrations occur in which energy range?**

- a) UV region
- b) Visible region
- c) Infrared region
- d) Microwave region

**2. The harmonic oscillator model is used to describe:**

- a) Rotational energy levels
- b) Translational motion of molecules
- c) Vibrational energy levels
- d) Electronic transitions



## Notes

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#### **3. What is zero-point energy in the context of molecular vibrations?**

- a) The energy at absolute zero temperature
- b) The minimum vibrational energy a molecule can have
- c) The energy required to excite an electron
- d) The potential energy at equilibrium

#### **4. The Morse potential curve differs from the harmonic oscillator model because it:**

- a) Accounts for anharmonicity in vibrations
- b) Ignores the effects of bond stretching
- c) Only applies to diatomic molecules
- d) Assumes energy levels are equally spaced

#### **5. The P, Q, and R branches in vibrational-rotational spectroscopy refer to:**

- a) Absorption lines of rotational spectra
- b) Transitions in electronic spectra
- c) Different modes of vibrations
- d) Overtones of vibrational modes

#### **6. What is the significance of the Born-Oppenheimer approximation in spectroscopy?**

- a) It treats nuclear and electronic motion separately
- b) It simplifies molecular orbital theory
- c) It assumes that vibrational and rotational energies are identical

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d) It explains the breakdown of molecular symmetry

**7. Which of the following factors affects the vibrational frequency of a bond?**

a) Mass of the atoms involved

b) Bond force constant

c) Both a and b

d) Only bond length

**8. Normal mode analysis in polyatomic molecules helps determine:**

a) The number and type of vibrational modes

b) Electronic energy levels

c) The molecular weight of a compound

d) Ionization energy

**9. Fourier Transform Infrared Spectroscopy (FT-IR) is preferred over dispersive IR spectroscopy because:**

a) It requires no sample preparation

b) It is faster and provides better resolution

c) It eliminates the need for a monochromator

d) Both b and c

**10. Metal-ligand vibrations are typically studied in which region of the infrared spectrum?**

a) Near-IR

b) Mid-IR



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c) Far-IR

d) UV-Vis

#### Short Answer Questions

1. What is the significance of the harmonic oscillator model in IR spectroscopy?
2. Explain the concept of anharmonicity and its effect on vibrational energy levels.
3. How does the Born-Oppenheimer approximation simplify molecular spectroscopy?
4. What is the difference between P, Q, and R branches in vibration-rotational spectroscopy?
5. Why are selection rules important in infrared spectroscopy?
6. What are normal modes of vibration, and how are they determined for polyatomic molecules?
7. How does the presence of overtones affect IR spectra?
8. What is the purpose of normal coordinate analysis in metal-ligand vibrations?
9. How does FT-IR differ from conventional IR spectroscopy?
10. List three major applications of infrared spectroscopy in chemical analysis.

#### Long Answer Questions

1. Describe the linear harmonic oscillator model and its limitations in infrared spectroscopy.



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2. Explain zero-point energy and discuss its role in vibrational spectroscopy.
3. Discuss anharmonicity and how the Morse potential diagram improves the understanding of molecular vibrations.
4. Explain the formation of P, Q, and R branches in vibration-rotational spectroscopy with an example.
5. Describe the selection rules for vibrational transitions and their effect on IR spectra.
6. Explain the concept of normal modes of vibration and their determination using group theory.
7. Discuss the significance of metal-ligand vibrations and their study in the far-infrared region.
8. What is the fingerprint region in infrared spectroscopy, and why is it important?
9. Explain the working principle of Fourier Transform Infrared Spectroscopy (FT-IR) and its advantages over traditional methods.
10. Discuss the applications of infrared spectroscopy in environmental, pharmaceutical, and forensic analysis.



## PHOTOACOUSTIC AND RAMAN SPECTROSCOPY

### Unit -10 Photoacoustic Spectroscopy (PAS)

Introduction photoacoustic spectroscopy. Photoacoustic Spectroscopy (PAS) is a powerful new analytical technique combining the principles of optical and acoustic spectroscopy. The process of PAS involves having a modulated light (not a continuous wave) source absorbed by a sample, which generates thermal energy that subsequently produces sound<sup>49</sup>. By analyzing these sound waves, one can obtain information regarding the sample's absorption characteristics, chemical composition, and structural properties. Overall, the advantage of PAS is its capability of yielding ultrasensitive measurements with nearly no treatment of the sample and hence it is widely used in many applications, such as pollution detection, chemical sensing, material sciences and so on. The core concept behind PAS is the photoacoustic effect, which describes light absorption by a material that rapidly heats the material and induces pressure wave traversing (acoustic wave) (Fig. 1). This is especially true for energies that are not efficiently dissipated as heat, which instead manifest as an acoustic signature that can be measured. By examining how frequently, intensely, and at what times this auditory signal occurs, scientists can determine many properties of the sample, including its chemical makeup, molecular framework, and surface properties. PAS offers significant advantages over traditional absorption spectroscopy, as it can allow for the acquisition of absorption spectra through opaque or highly scattering media, where the sample need not be transparent. The method is also able to analyze both bulk and surface properties of a sample, allowing it to be utilized on a wide variety of types of materials. Moreover, PAS yields high sensitivity due to the possibility of tuning the frequency of the modulated light to certain absorption wavelengths of the sample.

### **Basic Principles of PAS**

Photoacoustic Spectroscopy (PAS) is based on the interaction between modulated light (usually from a laser) and the sample, resulting in the production of an acoustic signal. The process starts with modulated beam of light, usually a laser, that hits

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the surface of the sample. The sample absorbs light, and it converts its absorbed energy to heat, according to the material. If the absorption is strong enough, it can lead to a rapid increase in temperature in the material, which when the sample expands brings a pressure wave. The pressure wave is what PAS detects/analyzes.

**Modulated Light Source:** A light source commonly, a pulsed or modulated laser shines onto the specimen. The light source can be adjusted to certain wavelengths depending on the material one wants to study. The power of the light is modulated, typically a square wave or sinusoidal modulation, often a periodic signal. The modulation enables the examination of the absorption characteristics of the sample across various frequencies. **Light Absorption;** Once the light comes into contact with the sample, sample absorbs energy. The degree of absorption is governed by materiality and absorption spectra. In the case of organic materials, the absorption can be because of electronic transitions, however, in other materials it may be because of vibrational or rotational transitions.

1. **Absorption of Light:** The incoming light energy is absorbed by the sample which gets heated up. Depending on the sample itself and its absorption properties, it can heat uniformly or in localized spots. The material will then expand due to heating.
2. **Sound Wave Generation:** With the ultra-fast heating, the material expands and contracts, producing a pressure wave, or sound wave. This is the photoacoustic effect, and the frequency and amplitude of the sound wave depend on the amount of absorbed light and the thermal properties of the sample.
3. **Acoustic Signal Detection:** The pressure wave or acoustic signature is subsequently detected by a highly sensitive microphone or piezoelectric transducer positioned in close proximity to the sample. A specific acoustic phenomenon is observed as a result of light being absorbed in various samples, allowing for spectrometry and other analyses based on the association between the intensity of the absorbed light and the detected signal.





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4. Filters: Different types of filters may be applied to concentrate on specific frequency ranges. For example, low-pass filters can enhance sensitivity by removing high-frequency noise, while band-pass filters can isolate features of interest. The accuracy and quality of the spectral data can be determined from the filter settings. Reconstructions; Using known data about the medium characteristics and the signal, reconstructions can be made to obtain images with multi-dimensional data (3D or 2D) using reconstruction algorithms. The Fourier transform is one way of converting the time-domain signal (the captured acoustic wave) into the frequency-domain, which can be compared with the known absorption spectra's of materials.

One of the most prominent features of PAS is its capability to achieve both bulk and surface sensitivity. The penetration depth of light varies with both the wavelength used and the optical properties of the material. Longer wavelengths, for example, may penetrate deeper into opaque or scattering materials, while shorter wavelengths are more surface sensitive. Additionally, PAS can be used in conjunction with other techniques, like ultraviolet-visible (UV-Vis) absorption spectroscopy, to obtain additional insights into a material's properties.

#### **Pros of Photo acoustic Spectroscopy**

**High Sensitivity:** High sensitivity is one of the most important reasons for using PAS. Because little material is needed to generate a measurable acoustic signal from the absorbed light, the technique can detect very low quantities of material. This characteristic makes PAS highly advantageous for trace analysis, which focuses on low concentration species. Non-destructive Technique; One of the key advantages of PAS is that it is a non-destructive technique, which means that the sample is not damaged or altered during the analysis. This is especially crucial in the investigation of fragile materials or when the sample needs to stay whole for subsequent examinations. Results are not dependent on the transparency of samples; PAS can be performed on opaque or scattering samples which is not the case with traditional absorption spectroscopy where samples must be transparent to the light. In addition, PAS can be employed to analyze a variety of

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sample types (powders, liquids, solids) that are often difficult or impossible to analyze using traditional absorption spectroscopy.

1. Bulk vs Surface Sensitivity: PAS can access both the bulk and the surface region of materials, depending on light wavelength. This is especially advantageous when investigating heterogeneous materials, such as composites, coatings, or multi-layered system.
2. High resolution: PAS works on the principle of modulation of the light source and the analysis of resulting acoustic signal which leads to high spectral resolution. It enables investigation of high-resolution vibrational modes and electronic transitions for materials having more insight into the properties of materials.
3. Broad Applicability: PAS can be used in a wide variety of environments, including gases, liquids, and solids. It is that is appropriate for studying complex systems including biological tissues, environmental samples, and materials science.

**Gaseous and condensed system applications**

In the case of photoacoustic spectroscopy, it has been elegantly been used for gaseous as well as condensed systems, thus making it a versatile tool for numerous scientific and industrial applications.

**Gaseous Systems**

In case of gaseous systems, PAS is commonly deployed for the absorption spectra studies in the infrared range. When modulated light passes through a sample of gas, each gas molecule absorbs specific wavelengths of light that match the energy and frequency of the molecules' vibrational transitions. The presence of these absorption features may be monitored through the detection of induced photoacoustic signals, and analysis of these signals yields key details about gas molecular structure, concentration, and identity. Detection of trace gases in atmospheric studies is one of the significant applications of PAS in gaseous systems. The method used can detect gases at very low concentrations, often at parts per



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billion (ppb) levels. This sensitivity is what makes PAS particularly appealing for application in environmental monitoring (detecting pollutants/greenhouse gases like CO<sub>2</sub>, CH<sub>4</sub>, or NO<sub>x</sub>). Another application of PAS is in pollutants monitoring, where it can detect harmful gases that are released in the atmosphere from industrial processes, transportation, and burning. The real-time monitoring provided by PAS is a valuable asset for compliance to environmental regulations and reduced impact of air pollution. PAS has an application not only in environmental monitoring but in chemical analysis of gases. In the example of a gas-phase reaction study, PAS can monitor the concentration of reactants and products involved in a chemical reaction. This ease to various reaction kinetics and mechanism comprehension.

#### Condensed Systems

PAS has been used to study absorption and thermal properties of condensed systems (liquids and solids). Because condensed materials typically have different light absorption wavelengths compared to gases, PAS is especially useful in studying solids and liquids' electronic and vibrational spectra. For instance, employing this technique in solid materials (semiconductors, metals and insulators) it allows to investigate the vibrational modes of the atoms in the material. This gives useful insight of the crystal structure, bonding and defects in the material. PAS can also be employed in liquid systems to investigate the molecular vibrations of liquids and solutions. This method is useful for the study of solvation effects, solvent-solute interactions, and chemical reaction dynamics in solution. Notably PAS has been widely used in studying biological systems (for example protein-ligand interactions, and the pharmaceutical industry, where it can aid understanding of drug solubility as well as formulation properties. PAS is also applied to material characterization. By analyzing PAS data, researchers can gain valuable insights into processes related to the molecular structure and degradation of polymeric materials, guiding the design and optimization of materials used in various industrial applications.

**PHOTOACOUSTIC  
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SPECTROSCOPY****Formulations for Chemical and Surface Applications**

Photoacoustic spectroscopy is especially good for a wide range of chemical properties and surface properties of materials. PAS is also a powerful technique to investigate heterogeneous system, because it can yield information about both bulk and surface properties.

**Surface Studies**

PAS is widely used in materials research for surface characterization, especially for thin films, coatings, and surface treatments. Because the surface may have different bonding and structure than the bulk material, surface-specific vibrational modes can often be detected with this technique. The surface of a material, for example, may generate distinct absorption features because of adsorbed or defect molecules.

**Unit -11 Raman Spectroscopy**

Raman Spectroscopy, a fundamental technique widely employed in the fields of analytical chemistry, physics, and material science, offers molecular level information about vibrational, rotational, and other low-frequency modes of a system. Raman spectroscopy, unlike infrared spectroscopy, measures the scattering of light so it gives complementary information on the molecular composition of a sample. The technique is named for the Indian physicist C.V. Raman, who first described the phenomenon in 1928 and won a Nobel Prize in Physics in 1930 for it.

**Quantum and Classical Theories of the Raman Effect**

Before diving into the basic principle of Raman Spectroscopy, it is crucial to gain insight into the Raman Effect, which underpins this phenomenon, as well as its theoretical underpinnings; classical and quantum. Jayesh Kumar, Waste Management Division, UCLA Department of Civil and Environmental Engineering. Electromagnetic radiation and interaction of light with matter are at the base of classical theory of Raman effect (Figure 2). According to this theory, when mono-



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chromatic light (most commonly laser light) is scattered by a molecule, there will be a shift of the frequency of the scattered light based on the vibrational or rotational modes of the molecule. It is these frequency shifts that we see as the Raman effect. When light falls on a molecule, it interacts with the electrons of the molecule. If the frequency of the light is equal to one of the vibrational modes of the molecule, some of the energy is absorbed and the molecule moves to a higher vibrational state. (So the light gets scattered, and then the scattering gets shifted to a lower frequency). This scattering is known as Stokes scattering, since the energy lost to the molecule means that the scattered light has a lower frequency. For example, in anti-Stokes scattering, the molecule is already excited in a vibrational state before scattering occurs. The absorbed energy by the incoming photons from the molecule is what results in a redshift of the scattered light. The classical treatment comes from polarizability, the degree to which the electron cloud surrounding a molecule distorts in response to an electric field (here, the electric field of the incident light). When the incident light interacts with the molecule and changes the polarizability of the molecule, scattering occurs; this is the Raman effect. This classical theory is enough in itself to explain the essential features of the Raman Effect, but it cannot account for all the details of molecular scattering.

#### **Quantum Theory of the Raman Effect**

Though the Raman effect is explainable through classical theory, the use of quantum theory offers a more comprehensive and precise framework to understand it. Based on quantum mechanics, molecules are made of discrete energy states, including rotational and vibrational energy levels. When light hits a molecule, it can cause transitions between these levels, but this interaction always involves the absorption or emission of quanta of light, the photons, and is thus governed by the rules of quantum mechanics. Beginning with the concept of virtual states, one can describe a quantum explanation for this phenomenon known as Raman scattering. In quantum language, when a photon meets a molecule, it excites the molecule to a virtual, transient state that doesn't correspond to any real energy level of the molecule. The molecule then relaxes from this virtual state by either emitting or absorbing a photon. The frequency shift of the scattered light is

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determined by the energy difference between the initial and final states (the vibrational states of the molecule). The electromagnetic field of the incident photon interacts with the polarizability of the molecule and produces Raman scattering. This interaction is quantified in quantum terms and corresponds to the change in energy states of the molecule, resulting in a shift of the frequency of the diffused photon. Additionally, the quantum theory includes selection rules, rules that state which transitions are permitted and how likely they are to occur.

**Inherent Rotational, Vibrational, and Vibro-Rotational Spectra**

Raman is especially good for vibrational and rotational states of molecules. These correspond to different classes of molecular motion, each producing distinctive features in the spectrum. In order to understand the spectra obtained in Raman spectroscopy, it is essential to understand the three types of spectra that can be generated as pure spectra, which are pure rotational spectra, vibrational spectra, and vibro-rotational spectra.

**Pure Rotational Spectra:**

Pure rotational Raman spectra are transitions between rotational energy levels of molecules. Such transitions are hence common in gaseous molecules possessing well-described ghf states corresponding to molecular rotation. The lower rotational states are populated at room temperature, so rotational transitions are highly sensitive probes of molecular interactions and can be detected on a short time scale (ns) due to the energy difference between rotational states corresponding to a shift in scattering frequency. Generally, the rotational Raman spectra are shown for top in linear or symmetric shape molecules. Low-temperature spectra are the most common since rotations need less energy than vibrations, so that the population of excited rotational states is low at high temperatures. Hence from the rotational Raman spectra, one may extract the moment of inertia and symmetry of the molecule. In order for rotational Raman scattering to occur, the molecule must have a permanent dipole moment which facilitates the interaction with the incident light's electromagnetic field. The changes in frequencies seen in the spectra



are related to the rotational constants of the molecule and give bond lengths and other structural information.

#### **Vibrational Spectra:**

The public domain Vibrational Raman spectra come from the transitions between various vibrational energy levels within the molecule. If the interaction leads to dipoled electronic transitions, it is possible for the energy quantum of the incident light to be equal to the energy difference between two vibrational states, which often results in the scattering of the light (Raman spectroscopy), which occurs in the mid-infrared spectrum. Molecules can stretch, bend, and rotate in multiple ways, but each type of vibrational motion has a frequency that is determined by the mass of the atoms involved and the bond strength between them. Stokes and anti-Stokes bands are usually observed in Raman spectroscopy due to vibrational transitions. Stokes bands are produced when the molecule gains the energy of the incident photons and goes to a higher vibrational state. Anti-Stokes bands, on the other hand, happen when the molecule starts in an excited vibrational state, and the vibrational energy is transferred to the scattering photon, giving a higher frequency of the scattered light. We consider the final result as the reference where we can obtain comprehensive information about the vibrational modes of the molecule including the frequency of each mode and the coupling of different modes are represented from the Raman spectrum. Useful for understanding molecular structure, bond strength, and chemical reactivity. Moreover, Raman spectroscopy is able to what is known as segmental substitution of the same molecule, ultimately providing information regarding isotopic substitution and examining molecular dynamics.

#### **Vibro-Rotational Spectra:**

Raman spectroscopy detects vibro-rotational spectra besides pure rotational and vibrational spectra as they are simultaneous transitions that helps to study the vibrational and rotational energy states of molecules. As such, vibro-rotational transitions are of particular importance for the study of polyatomic molecules, for which considerable coupling between the rotational and vibrational motions takes

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place. The reason these spectra appear is that the rotation and the vibration of the molecule are coupled. Because spectra are influenced by both the molecular rotation and vibration, vibro-rotational spectra have more complicated shapes than vibrational spectra. These transitions usually involve a change in both the rotational quantum number and vibrational quantum number, allowing probing of the coupled dynamics of the molecule. The vibro-rotational raman spectra also give us in-depth information about the rotational-vibrational coupling, the geometry of the molecules, the interaction of various vibrational modes with each other etc. So this spectroscopy is generally used in complex molecules studies including biological systems, complex organic molecules, and molecular assemblies.

**Dependent Selection Rules and Mutual Exclusion Principle**

It is called selection rules in Raman spectroscopy referring to the rules which govern the allowed vibrational or rotational transition. They are derived from the symmetry of the molecule and its interaction with the electromagnetic field of the incident light. The selection rules provide vital information about possible transitions in a scattering process and for understanding the Raman spectrum. Raman spectroscopy is an important and widely used technique to explore vibrational, rotational and other low-frequency modes in molecules. Raman spectroscopy definition revolves around the selection rules: understanding what vibrational or rotational transitions are observable in the Raman spectra. These selection rules determine when a molecule will scatter light through the process of Raman scattering. Specifically, three general selection rules identify Raman active vibrational modes: the change in polarizability of the molecule, vibrational symmetry of the normal mode, and conservation of energy. The interaction of all of these factors plays an important role in dictating whether or not this type of molecular vibration will result in observable Raman scattering and, subsequently, if it can be detected by Raman spectroscopy. In this post, we shall delve into these selection rules in more detail and explain their importance and role in the Raman scattering process.

**Change in Polarizability**





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First and foremost requirement for the selection rule in Raman scattering is that the polarizability of the molecule must change as it goes through vibrational or rotational motion. Polarizability of a molecule describes how readily its electron cloud will be distorted by an external stimulus like an electric field, for instance, that introduced by the process of light impinging on the molecule. Raman scattering is thus possible because the polarizability of the molecule changes during its vibrational motion. In other words, Raman active means the oscillation of the atoms when vibrating causes a change in the electron cloud of the molecule. If the vibration causes a temporary distortion of the electron cloud, which changes the polarizability, then the molecule can interact with incident light. This interaction results in the scattering of the photon, and the energy dispersion of this scattering process contributes to the formation of what we call the Raman spectrum. This rule means that only those vibrational modes that change the polarizability will cause Raman scattering. Some vibrational modes, for example those involved in bond stretching and bending, will induce an electron cloud displacement with bond compression or elongation. These modes are Raman active as they result in a change in the polarizability of the molecule. Conversely, vibrational modes that lead to no change in the electron cloud or the molecular polarizability are Raman inactive. For example, symmetric stretching or bending motions in which the overall electron distribution remains invariant would have no observable Raman scattering. To help visualize this, think of a molecule with symmetrical geometry, such as a homonuclear diatomic molecule ( $O_2$ ,  $N_2$ , etc.). If the molecule vibrates symmetrically, like if it stretches, both atoms would move in and out together, which may not change the polarizability of the molecule enough to produce a Raman signal. But if the molecule undergoes an asymmetric stretch (the two atoms move in opposite directions), the polarizability will change and cause Raman scattering. Therefore, the type of molecular vibration is very important in disrupting the polarizability to the extent to cause Raman scattering. Essentially, this selection rule dictates that any vibrational modes which correspond to a stretching or bending of bonds through distortion of the electron cloud are Raman-active. On the other end, internal movements like torsions or

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rotations, which do not impact the polarizability of the molecule, are typically not Raman active.

**Symmetry of Vibrational Modes**

Another important selection rule for Raman scattering is the symmetry of the molecular vibrations. Whether a vibration can induce change of the polarizability detectable by the incident light is determined by the symmetry with respect to the vibrational modes of the molecule. To determine whether a vibration will be Raman active, you need to analyze the molecular symmetry. Symmetry analysis is a method used, passed on the group theory (branch of mathematics) around the symmetry analysis of molecules. Group theory assists in classifying the symmetry properties of different molecular vibrations, including how they behave for rotations and reflections. For a molecule, by applying group theory to its vibrational modes, one can ascertain which modes will give rise to a change in polarizability and which will not. To be Raman active, then, the vibrational mode must belong to a specific symmetry species that permits it to couple with the incident light. So the vibration has to produce a change in polarizability that can couple with the electric field of the incoming light. Whether this interaction can happen, depends on the symmetry of the vibration. Particular example of this arise when a certain symmetry species within a molecule's vibrational modes are Raman active, as the change in polarizability they induce is symmetry allowed. Take any simple molecule, for example carbon dioxide ( $\text{CO}_2$ ), a linear molecule which possesses high symmetry. Vibrational modes of the molecule can be grouped based on their symmetry properties.  $\text{CO}_2$  bending modes are Raman active, as they involve change in the polarizability of the molecule. On the other hand, some modes e.g. symmetric stretching mode does not change polarizability and would be Raman inactive. However, in molecules or systems with lower symmetry, it becomes more crucial to consider the selection rule from symmetry. In more complex molecular structures, vibrational modes must be analyzed in detail to see whether they are Raman active. For example, in an asymmetric molecule, some vibrations can be Raman active while others do not conform to symmetry requirements for inducing detectable changes in polarizability.



#### Energy Conservation

As with all spectroscopic techniques, Raman scattering adheres to the law of conservation of energy, which dictates that the sum of energy in the system before and after a scattering event must remain constant. As a result, the energy difference between the starting and ending slots of the molecule is preserved throughout the scattering process. For example, in Raman scattering, energy conservation means that the energy difference between the incoming photon and the outgoing photon must equal the energy of the molecular vibration. This is both Stokes and anti-Stokes scattering events.

#### Stokes Scattering

Raman scattering in the form of Stokes scattering is one of the two main processes of Raman spectroscopy. That's when the incident photon interacts with the molecule and passes on part of its energy, making the molecule move to a higher vibrational state. Consequently, the scattered photon leaving the molecule will have lower energy than the incoming photon. This energy loss of the photon matches the energy difference between the initial and final vibrational states of the molecule, representing the signature of Stokes scattering.

#### Stokes scattering proceeds as follows:

Molecule sits in its ground vibrational state. When one of the laser photons hits the molecule, the photon transfers some of its energy to the molecule, exciting it to a higher vibrational state.

5. Energy Transfer: The energy gap between the ground state and excited vibrational state, is transferred from the photon to the molecule. Because of this interaction, the photon loses energy.

6. The Scattered Photon: After this interaction, the photon leaving the molecule as a scattered photon has lower energy than the incoming photon. The

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difference in energy between the incident and scattered photons is associated with the vibrational transition in the molecule.

7. Stokes Shift: The difference in energy between the scattered and incident photon leads a shift toward longer wavelength (lower frequency) in the Raman spectrum. This characteristic is known as the “Stokes shift,” and it is a hallmark of Stokes scattering.

That gives the Stokes shift information on the vibrational modes of the molecule. Scientists analyze the wavelength or frequency of the Stokes-scattered light, it gives them information on molecular structure and the chemical composition of the sample and which types of molecular vibrations are taking place in the sample. Moreover, temperature, concentration, and the type of molecular interactions also determine the magnitude of Stokes peaks in the corresponding Raman spectrum.

**Anti-Stokes Scattering**

Anti-Stokes scattering, the opposite of Stokes scattering, takes place when the photon emitted has more energy than the incoming one. This is when the molecule is first in an excited vibrational state. In this case, the molecule loses energy to the incident photon, so the photon scatters at a higher energy than it originally had. The increase in energy of the emitted photon corresponds to the energy difference between the vibrational ground state and the excited vibrational state of the molecule, and appears as an anti-Stokes shift in the Raman spectrum.

**Anti-Stokes scattering process can be explained as follows:**

Excitation of the molecule: Not in its ground vibrational state. In contrast, it is already in an excited vibrational state (above the ground state). This happens because of former thermal excitation, in which the molecule has absorbed from the environment energy and in a higher vibrational state.



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1. Photon to Energy Transfer: When photon hits the molecule, energy from the molecule is transferred to the photon. The reason is that the scattered photon has a greater energy than the incoming photon.
2. The scattered photon: as a consequence of scattering, it has more energy than the incident photon, and the energy difference between the incident photon and the scattered photon corresponds to the energy difference between the initial and final vibrational states of the molecule.
3. Anti-Stokes Shift: The subsequent increase in energy of the scattered photon causes a shift in the Raman spectrum towards shorter wavelengths (higher frequency), known as the “anti-Stokes shift.”

Anti-Stokes scattered light is less intense than Stokes scattered light in general. This is because at room temperature, there are fewer molecules in higher vibrational states than in the ground state, so there are less chances for anti-Stokes scattering to happen. For example, the relative intensities of the Stokes and anti-Stokes peaks can reveal the sample temperature, since the population of molecules in excited vibrational states is a function of the temperature.

#### **Raman Spectroscopy: Stokes and Anti-Stokes Scattering**

Stokes and Anti-Stokes scattering are central to the Raman effect, which is utilized in Raman spectroscopy to ultimately deduce vibrational information about the molecule. Raman spectrum generally has Stokes and anti-Stokes components, with Stokes scattering is usually the larger component of this spectrum due to the larger number of molecules in the vibrational ground state.

#### **Related effects of temperature and the Stokes/Anti-Stokes Intensity Ratio**

Thermodynamic information can be gleaned from the ratio of the intensities of the Stokes vs. anti-Stokes peaks. The distribution of molecules in various vibrational states (anti Stokes and Stokes scattering) follows a Boltzmann distribution, and there is always a population in an excited vibrational state, but it decreases with temperature. As a result, at higher temperatures, the intensity of anti-Stokes peaks increases as a larger fraction of molecules reside in excited states. At lower

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temperatures, more molecules can be found in the ground vibrational state, giving rise to Stokes peaks. Depending on the energy levels of the vibrational states and the temperature of the system, the ratio of the Stokes to anti-Stokes peak intensity can be related to the temperature of the sample and, therefore, the relative intensities may be used for such a measurement.

**Stokes and Anti-Stokes Scattering Applicability**

**Molecular Identification:** The detailed vibrational spectra acquired from both the Stokes and anti-Stokes scattering are utilized in molecular identification.

This result in peaks that represent the absorbance of the various bonds within a compound, and, by studying the displacements and strengths of these peaks, scientists can identify the chemical bonds and functional groups within a sample.

1. **Material identification:** The Stokes and anti-Stokes shifts present in the Raman spectra are extremely helpful in identifying the materials whether polymers, nanomaterials, thin film, etc. The spectral characteristics help to probe into the molecular structures, chemical compositions as well as mechanical properties of the materials.
2. **Thermal Analysis:** As indicated previously, the relative intensities of the Stokes and anti-Stokes peaks are temperature sensitive. By well understanding these intensities, scientists can learn about the thermal properties of materials and study the thermally induced molecular vibrations.
3. **Pharmaceutical and biomedical applications:** Raman spectroscopy, especially the usages of Stokes and anti-Stokes scattering, finds potential application in the pharmaceutical industry for the analysis of drugs, monitoring of purity of the pharmaceutical products, and studies of drug–biomolecule interactions. At the same time, within biomedical research, Raman spectroscopy benefits tissue and cell studies, offering precise chemical profiles that improve disease detection or biological processes monitoring.



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4. Environmental sensing: Raman spectroscopy, owing to the Stokes and anti-Stokes scattering, is also employed for environmental monitoring, such as the identification of pollutants and greenhouse gases. Both scattering techniques allow for the sensitive detection of numerous chemical compounds in air, water, and soil matrices, contributing to environmental pollution monitoring and mitigation efforts.

This conservation of energy guarantees that the energy of the incident light is matched by the energy gained or lost from the molecule through the scattering event. This principle also governs the shape of the Raman spectrum, specifically the relation between Stokes and anti-Stokes peaks. The Stokes peaks are generally more intense since most molecules are in the ground state and the scattering probability is higher from the ground-state to the excited-state than from the excited-state to a higher energy state. In addition, energy conservation constrains the total Raman signals intensity. The Raman shift, i.e., the difference in the energy of the incident and the scattered photon must match the molecular vibrational energy levels. Because the energy quantized rules determined by the molecule, that means vibrational energy levels must be quantized, thus the scattering events will only occur for certain vibrational modes with energy differences corresponding to the energy of the incident photons.

#### **Mutual Exclusion Principle:**

According to the mutual exclusion principle in Raman spectroscopy if one of a vibrational mode is IR (infrared) active than Raman inactive or vice versa. This is the consequence of the fact that for a mode to be IR active there should be a change of molecular dipole moment during the vibration, whereas for a mode to be Raman active, the polarizability of the molecule should change. Thus, IR active modes (the ones that induce

#### **Unit -12 Advanced Raman Techniques**

Raman Spectroscopy, a technique that measures the scattering of light from gas molecules (due to molecular vibrations), is now an invaluable tool in a multitude of different fields from material science and chemistry to biology. Conventional

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Raman spectroscopy offers a deep insight into molecular vibrations and interactions, whereas advanced Raman techniques improve the sensitivity, resolution, and scope of applications of this technique. These advanced techniques include Resonance Raman Spectroscopy and Coherent Anti-Stokes Raman Spectroscopy (CARS), both of which provide unique advantages for probing molecular structures and dynamics. These approaches extend the limits of conventional Raman spectroscopy, giving investigators the ability to see fine details in materials that might be difficult to analyze otherwise.

**Resonance Raman Spectroscopy**

Resonance Raman Spectroscopy (RRS) - RRS is an advanced Raman technique where the Raman scattering signal is amplified using the resonance effect between the monochromatic laser light and target molecule electronic transition or vibrations. In fact, the major concept of resonance Raman spectroscopy is that when the frequency of incident light coincides with a molecular electronic transition, the scattering efficiency is significantly enhanced. This resonance effect enhances the intensity of the corresponding spectral features, significantly improving the sensitivity of the detection of those specific vibrational modes, which is the reason why resonance Raman spectroscopy is such a powerful tool, particularly in the analysis of systems that possess any suitably weak or poorly detectable vibrational modes in comparison to other modes, which can hamper study using general Raman techniques. The resonance effect is when the frequency of the incident laser light closely matches an electronic transition of the molecule, causing the system to “resonate.” Now when the molecule is placed in the vicinity of the nano-material, the energy of the incoming photon is selectively absorbed and scattered by this molecule, leading to an enhancement of the Raman signal. This effect is a result of electronic-vibrational coupling as the vibrational transitions are coupled to the electronic excitations of the molecule. The scattering process in conventional Raman spectroscopy happens between an incident photon and the vibrational energy levels of a molecule, with the shift in frequency indicating the energy difference between the initial and final vibrational states. But in the case of resonance Raman spectroscopy, the incoming photon is in resonance with an





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electronic transition, contributing to the intensity of the scattered photons and hence the Raman signal. This leads to vibrational bands that are much more pronounced, allowing researchers to probe vibrational modes that would typically be too weak to observe. Resonance Raman spectra are usually measured using a laser source, the frequency of which is near, or coincides with an electronic transition of the molecule. For example, the laser is often appropriate to the particular absorption bands or optical transitions associated with the molecule of interest. Sensitive Raman signals are especially valuable for studying chromophores, or molecules with strong electronic absorption bands in the visible or ultraviolet range.

#### **Resonance Raman Spectroscopy and Its Applications**

It is also very important in the study of chromophores, which are molecules that absorb light at specific wavelengths and are accountable for the coloration of biological as well as chemical systems (Raman spectroscopy and its applications). Resonance Raman spectroscopy yields high sensitivity and selectivity because their high-power laser light resonantly excites the electronic transitions of these molecules and allows them to detect even the weak chromophore vibrational modes which are hard to observe by usual Raman spectroscopy. Biological and Biochemical Research: RRS is often used to probe biological systems, such as proteins, nucleic acids, and metalloenzymes. Many of these biomolecules harbored chromophores or metal centers that can be excited with resonance light, producing enhanced Raman signals that elucidate molecular structure, dynamics, and interactions. Atypical example for applying resonance Raman spectroscopy is the hemoglobin, with its heme group capable of a strong electronic absorption in the visible region. Environmental and Surface Analysis: RRS aids in observing and characterizing the surface characteristics of materials with localized electronic states, which includes, but is not limited to semiconductors, carbon nanotubes, and nanomaterials. In such cases, resonance Raman spectroscopy enables access to vibrational modes that can be strongly coupled to surface electronic states or defects enabling access to material electronic structure, strain and other characteristics. Resonance Raman spectroscopy can also be used to study reaction



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dynamics and mechanisms of chemical, in particular, it is used to study molecular dynamics and reaction mechanisms. Resonance Raman spectroscopy allows for measurement of vibrational modes coupled to electronic transitions in the excited states active in a reaction<sup>54,55</sup>, and thus can probe the molecular excited states involved in the reaction itself.

### Benefits of Using Resonance Raman Spectroscopy

Resonance Raman spectroscopy (RRS) is a very powerful and highly labeling free vibrational spectroscopic tool that is based on the enhancement of the Raman scattering cross-section by exciting the system close to an electronic absorption band. This improvement significantly increases the sensitivity and power of Raman spectroscopy and allows it to examine a wider variety of samples from low concentration species to complex biological systems. RRS offers all the access to subtle vibrational features necessary for understanding molecules' complexities on the electronic and dynamical scales by analysing the inelastic scattering of monochromatic light by molecules. Resonance Raman spectroscopy has a number of key benefits, which makes this technique highly relevant both in terms of fundamental research and applied sciences.

### Enhanced Sensitivity

This resonance effect leads to one of the biggest advantages of resonance Raman spectroscopy, an increase in sensitivity. In standard Raman spectroscopy, as scattered light is normally weak, background noise can dominate the Raman scattering signal. However, when we choose an excitation wavelength in resonance with a certain electronic transition of that molecule, the intensity of the Raman scattering increases in orders of magnitude. This improvement is due to the effective coupling of the incident light with the electronic states of the molecule, resulting in an enhanced probability of the Raman scattering from selective vibrational modes. Raman signal enhancement using resonance enables exclusive enhancement of certain vibrational modes, making weak vibrational features detectable that would be otherwise unobservable in non-resonance Raman spectroscopy. This is especially advantageous when analyzing molecules found in low concentrations,



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like trace analyses in complex matrices. In. vibrational modes that weak in Karn spectroscopy (Gurevich et al., 2019), such as some low-frequency resonances. Resonance Raman spectroscopy, for instance, is commonly applied where aromatic compounds, metal-ligand bonds, and conjugated systems exhibit high Raman enhancement when resonant with their electronic transitions. Implementing resonance Raman spectroscopy with increased sensitivity has transformative potential for a wide range of applications from environmental monitoring and material science to biochemistry, as the capability to detect incredibly minute quantities of analytes is often critical in these fields. In biological systems where molecules are often present in low concentrations, resonance Raman spectroscopy allows the detection of specific biomolecular such as proteins, nucleic acids, and lipids with little or no complex sample preparation or labeling.

#### Selective Probing

Resonance Raman has another important advantage that is selective enhancement of the vibrations, which gives to opportunity to probe specific molecular components in multicomponent systems. Since Raman's scattering is weak, the scattering from certain vibrational modes, and be selective and is often used in RRS to identify specific chemical bonds, functional groups, or molecular conformations allowing RRS to be a strong tool for differentiating between components in mixtures or complex biological samples. In case of complex mixtures, we can identify and examine the individual components based on their characteristic vibrational signatures using the technique of resonance Raman spectroscopy. This enables chemists to amplify certain resonances of interest while suppressing others, facilitating the study of the modes of interest relevant to functional groups, e.g., aromatic rings, double bonds ( $C=C$ ,  $N=N$ ), or metal-ligand bonds. For instance, in biological systems, resonance Raman spectroscopy may selectively enhance the vibrational modes of individual biomolecular such as proteins, DNA, and lipids, enabling distinctions that may be difficult to discern with conventional Raman spectroscopy. This selectivity is especially useful when analyzing more complex sample types like cell lysates, tissue extracts, or blood where many molecular components are present at the same time. Additionally,

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resonance Raman spectroscopy yields molecular site-specific insights into molecular environment, including the coordination of metal ions in metalloproteins or the conformation of nucleotide bases in nucleic acids. Understanding the behavior of individual molecular components within a complex system is critical to many fields, giving the 'super planar' information valuable applications in structural biology, drug development and materials science. By only probing specific vibrational modes, the selective interrogation also allows for a more efficient analysis of the data. Resonance Raman spectroscopy can minimize complex overlap with the use of specific molecular features, thus simplifying the analysis of complex systems, which can prove to be both less cumbersome and time-consuming.

**Non-destructive Analysis**

As with conventional Raman spectroscopy, one of the main advantages of resonance Raman spectroscopy is its non-destructive character. Because resonance Raman spectroscopy is based on scattering of the light from the sample rather than the absorption or emission of photons by the sample, it does not necessitate any chemical alteration or destruction of the sample. Its label-free nature makes RRS extremely useful for studying sensitive or expensive samples such as rare biological specimens, historical artworks, and thin films of modern materials.

In biological applications, resonance Raman spectroscopy minimizes changes to the sample itself, enabling repeated measurements to be made without altering its structure or properties. This is especially crucial in live-cell, tissue or organism studies where the impacts of sample preparation or invasive methods (such as labeling) may alter the native system activity. One of those methods, resonance Raman spectroscopy has been widely used to investigate protein-ligand interactions, enzyme catalysis, and even cell biology without perturbing the tested biological system. Because it is non-destructive, resonance Raman spectroscopy can be used for real-time probing of chemical reactions or molecular interactions. The direct observation of a particularly sensitive vibrational transition can yield information about the kinetics of reactions or other critical processes on the fly,



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opening a door to applications in areas such as drug screening to monitor how a candidate drug scaffold interacts with a biological system without alteration.

#### More Spectral Information

Resonance Raman spectroscopy allows for significantly more spectral detail than traditional Raman spectroscopy. If the excitation wavelength is tuned into resonance with an electronic transition of the molecule, the Raman scattering intensity for this mode is enhanced, as is the resolution of less intense or even hidden modes in ordinary Raman spectra. Such additional modes can yield important information about the molecular structure, electronic properties and dynamics of the system being studied. GaN) to further investigate excited-state dynamics, charge transfer, and electron-phonon coupling within molecular systems. Researchers can gain insights into the electronic structure, including energy levels, electron density distributions, and electronic-vibrational coupling, by examining shifts in spectral wavelengths and reversible or irreversible changes in the intensity of specific vibrational hot spots. This supplementary spectral information is particularly useful in analyzing conjugated systems (e.g., polyenes, proteins, and nucleic acids) and metalloproteins in which coupling between electronic structure and vibrational characteristics is pronounced. Spectroscopy is a technique in which light is used to probe the coordination environment around the metal centers in metalloproteins, and the vibrational modes associated with metal-ligand bonding to give a greater insight into the function and mechanism of action of the protein. In addition, resonance Raman spectroscopy can be incorporated into multidimensional techniques, e.g. time-resolved resonance Raman spectroscopy or resonance Raman imaging, to monitor the dynamics of molecular systems in real-time or spatially resolved ways. Temporal or spatial maps of vibrational events within systems are presumably more detailed than otherwise obtainable via other methods, while offering previously unattainable resolution over both time and space over which molecules are operating.

#### Applications in Biological and Medicinal Chemistry

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Another area where resonance Raman spectroscopy has shown great potential is in the study of biological and medicinal chemistry, with applications ranging from the study of individual biomolecules to complex biological processes. The unique capability of this technique to selectively enhance certain vibrational modes and to probe molecular structure in multi-component, complex biological systems has made this an invaluable technique for studying proteins, nucleic acids (DNA and RNA), lipids, and drug interactions. The role of resonance Raman spectroscopy in protein studies is very important, given that it is frequently employed at the level of secondary and tertiary structures to assess biologically relevant structural variations, either induced by binding events or enzymatic activity. This helps researchers study topics like protein folding, dynamics and interactions by selectively amplifying the vibrational modes corresponding to a protein's backbone or certain amino acid residues which can give crucial biochemical insights to their biological functions. Resonance Raman spectroscopy has also been used to investigate the different conformational states of nucleic acids (like DNA and RNA) and their binding with proteins or small molecules. Resonance Raman spectra of DNA, for instance, yield insights into the stacking interactions between nucleotide bases, helix conformation, and the effects of ligand binding on the structure of the nucleic acid. As a result, resonance Raman spectroscopy is a powerful diagnostic tool for drug discovery and genetic studies, where an understanding of small molecule and nucleic acid interactions is critical for the rational design of therapeutic agents and gene control mechanisms.

Additionally, in the field of drug design and pharmacology, resonance Raman spectroscopy has been employed to investigate the binding of small molecules to their targets, including binding to receptors, enzymes, or membrane proteins. Indeed, by selectively enhancing the vibrational modes that are related to either the drug or to the target, researchers can achieve detailed information about the binding site, the binding affinity, and even the conformational changes that drug binding induces. It plays an essential role in modulating the pharmacological properties of drug candidates and elucidating the molecular mechanisms of action of existing therapeutics.



#### Applications in the field of materials science

In the field of materials science, resonance Raman spectroscopy is used to investigate semiconductors, nanomaterials, and carbon-based materials, including graphene and carbon nanotubes. The technique provides valuable insights into the electronic structure and vibrational dynamics of these materials, making it a powerful tool for the characterisation of advanced materials at the molecular level. For instance, in the research of graphene and other carbon nanomaterials, we could study the D-band and the G-band vibrations by employing resonance Raman spectroscopy, which are sensitive to the defects, dopants, and local ordering of the carbon atoms in the specimen. Such tools and techniques provide researchers with detailed information regarding the quality, functionality, and electronic properties of material, all of which is essential for applications in electronics, energy storage, and nanotechnology. In a similar vein, phonon modes and exciting behavior in semiconductors can be probed by resonance Raman spectroscopy, unlocking information about the optical properties of the material, the band structure, and charge transport characteristics. Such information is hugely important for the design of new materials for solar cell, light-emitting diodes (LEDs) and other optoelectronic materials. Resonance Raman spectroscopy (RRS), a well-established technique to study molecular vibrations, provides a significantly improved signal-to-noise ratio by utilizing the resonance between the incident light and the electronic transition of the molecule being monitored. RRS has many advantages when compared to traditional spectroscopic techniques, including greater sensitivity and selectivity of probing but also limitations, including the inability to efficiently study complex molecular systems. These restrictions may lead to reduced applicability of the technique and prevent it from providing adequate and precise structural information in some cases. Importantly, those employing resonance Raman spectroscopy should understand the limitations of the technique and should consider when and how to best apply it, and when other techniques may be better suited for the question at hand.

#### Resonance Conditions

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One key drawback of resonance Raman spectroscopy is that the incident light must resonate with the electronic transition of the molecule being studied. The incident photons be absorbed by the molecule through resonance, in which case their energy is close to the energy difference between the ground state and the excited electronic state of the molecule, for this to take place. When the energy of the incident light is not required to resonate with the electronic transition or close to it, the Raman scattering will not be enhanced (no resonance effect will occur), and the collected scattering signal will always be small (or even undetectable). While specificity in term of resonance conditions can be a serious stumbling block, especially for those molecules for which the electronic transitions of interest do not match with the excitation wave-lengths available in laboratory. In these cases, obtainment of improved Raman signals is very hard or even impossible, resulting in weak or insufficient spectral details. Additionally, the resonance conditions can be hard to satisfy, since an experimental step may deal with a complex molecule or a system with many overlapping electronic transitions, which yield ambiguities in spectral interpretation and lower specificity when trying to probe certain vibrational modes. If these means are not available, then we can revert to normal Raman spectroscopy or other spectroscopic techniques that do not provide the same level of sensitivity or specificity as resonance Raman does. Consequently, this hampers the general applicability of RRS for all the molecules or systems, particularly when the resonance conditions are challenging to satisfied.

**Overlapping Signals**

Resonance raman spectroscopy can show some overlapping signals limitations, especially when the peaks related to the enhanced electronic transition show such an intense dominance on the spectrum. This effect is known as resonance Raman scattering and it dramatically increases the intensity of the scattered radiation from particular bonding modes close to the electronic transition of the molecule. This is a useful improvement, but it can lead to the situation where certain vibrational modes are dominating over others, which can happen if the studied molecule has several vibrational modes with close in energy. This creates





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challenges particularly in cases where the vibrational modes are closely spaced or when the signal from the primary electronic transition drowns out the vibronic transitions. The spectra that result can be crowded and backgroundy and make it difficult to draw detailed information about the molecular structure or dynamics. And, at times, the enhanced peaks due to the resonance condition can even hide the fingerprints of weaker vibrational modes, which can be valuable to molecular unravelling or functional genes. This challenge is especially severe in the study of complex systems, or in molecules with several electronic transitions. The existence of multiple electronic bands in the excitation range can therefore give rise to a forest of resonantly enhanced Raman features that mask the more subtle vibrational signatures of interest. Consequently, resonance Raman spectra are not always pure and may be analyzed with higher scrutiny, i.e., powerful computational techniques or experimental work-arounds (modulating the excitation wavelength) would be needed in order not to confuse overlapping signals and withdraw meaningful information. If the resonance Raman spectrum is dominated by certain vibrational modes, one may use other spectroscopic techniques for confirming their findings or to obtain complementary data. In many cases, combining standard Raman spectroscopy, infrared (IR) spectroscopy, or other complementary techniques such as fluorescence spectroscopy would give more information on the molecular system without the interference of overlapping resonance-enhanced signals.

#### **20 Bioreactor Equipment at Commercial Scale<sup>18</sup>.**

Although resonance Raman spectroscopy is more sensitive than normal Raman spectroscopy in many cases, the resonance effect is also very sensitive to temperature, solvent effects, and impurities. Variations in these parameters can alter both the energy of the electronic transitions, which may result in changes to whether the resonance conditions are satisfied. Such dependence on the experimental environment can lead to difficulty in obtaining reproducible results in different conditions, particularly in heterogeneous or dynamic materials. This would change the resonance conditions if the solvent environment is influencing the electronic transition of the molecule, that would not be in conformance with the

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intensity and profile of the Raman peaks. Likewise, temperature changes can affect the vibrational frequencies and electronic states of the molecule, and therefore the resonance Raman spectrum. In the presence of biological or complex materials, movement and fluctuations in the surrounding environment can lead to spectral features to shift or broaden, thus making it difficult to interpret what is actually being probed in the measurement. In order to minimize these challenges, researchers usually go to painstaking lengths to control the experimental conditions temperature, choice of solvent, sample purity, etc. Nonetheless, the environmental sensitivity of resonance Raman spectroscopy finally presents an important caveat in systems facing dynamic or intermittent conditions and to the best of our knowledge all this has not been addressed so far.

**Instrumentation and Cost**

Resonance Raman spectroscopy necessitates specialized instrumentation to facilitate the appropriate resonance conditions, such as tunable lasers with specific excitation wavelengths that correspond to the electronic transitions of the molecular species being studied. Typically, this specialized equipment is costly and may not be present in common laboratory provisions. So the lack of such instruments may restrict the widespread application of the technique, for example in labs with limited resources or that do not often require high-resolution vibrational spectroscopy. Resonance Raman spectroscopy often demands elaborate experimental setups beyond just the price of the laser systems and detectors, in order to obtain the desired excitation wavelengths and preserve stable experimental circumstances. This could involve highly controlled conditions for the sample, such as stabilizing at a specific temperature or ensuring proper alignment of the sample with the laser beam. The extra demands of resonance Raman experiments could add complexity and expense, limiting its adoption in not so reasonable disciplines or some exploration. Furthermore, resonance Raman spectroscopy can depend on knowledge of how to obtain specialized instrumentation, interpretation of complex spectra, and the expertise of resonance enhancement. This specialized knowledge and skill set not only require thorough training but also may pose challenges for



researchers who are not experienced with the technique, restricting its use in various research domains.

#### **Restricted to Molecular Systems with Available Electronic Transitions**

Resonance Raman spectroscopy works best on molecules which have electronic transitions accessible to the excitation light. Not all molecules do, however, or have such fast and well-defined electronic transitions within reach of the excitation laser. For instance, larger more complex molecules or molecules that do not have clear absorption characteristic in the visible or ultraviolet (UV) spectral region may not lead to resonance enhancement and thus make RRS less effective for such systems. For a molecule with electronic transitions that are not rendered in the range of available lasers for excitation or with low energy transitions (instead of a high-energy former resonance Raman spectroscopy could not be used or can provide very little information. For example, small or non-aromatic molecules, in addition to having weak electronic transitions, may not experience enough resonance enhancements to be useful as spectral data. Conversely, systems that exhibit wide or ill-defined electronic bands might not display the necessary sharp features for resonant enhancement of Raman signal associated to enhanced Raman scattering, for which case traditional Raman spectroscopy may be the ideal choice. These limitations demonstrate proper needs for careful evaluation of the studied molecule or system before determining resonance Raman spectroscopy as the technique of choice. And, in situations in which resonance conditions are impractical to achieve, research may need to investigate other spectrometric techniques, such as conventional Raman, infrared or fluorescence spectroscopy, to obtain the requisite structural or vibrational details.

#### **Little Structural Information for Large and Complex Systems**

While it allows for significant visualizations of the vibrational modes of small and medium-sized molecules, resonance Raman spectroscopy is less suited to study large or highly complex systems, such as large proteins nucleic acids, or super molecular assemblies. This limitation can primarily be attributed to the complexity of the spectra produced by large molecules, which can have an extensive number

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of overlapping vibrational modes. Under such resonant conditions, the spectrum can become quite complex, with many enhanced and challenging-to-assign peaks, particularly when the sample contains clinically novel combinations of interacting components. The ER peaks in large biomolecular like proteins can correlate with contributions of individual parts of the molecule, making it difficult to separate individual vibrational modes. Despite resonance Raman spectroscopy yielding some useful structural information on certain aspects of large molecules (such as metal coordination or specific functional groups), it is not adequate for obtaining a global structural view of the entire macromolecule. To paint a more complete picture of the molecule's structural and dynamic character, researchers typically combine resonance Raman spectroscopy with numerous other techniques, including nuclear magnetic resonance (NMR) spectroscopy, X-ray crystallography or cryo-electron microscopy (cryo-EM).

**Challenges in Identifying Enumerated Vibrational Modes**

One more drawback of resonance Raman spectroscopy remains the difficulty in assigning the vibrational modes to specific molecular features or functional groups, especially under resonance conditions in the presence of many overlapping transitions. For resonance condition, the enhanced Raman peaks give rise to a congested spectrum, resulting in difficulties in resolving and identifying individual vibrational modes to bonds or molecular features. This is especially the case for molecules with complicated electronic structure or multiple functional groups. Since vibrational peaks sometimes overlap and provide complicated signals, researchers can be going for advanced computational methods, like density functional theory (DFT), to help in making assignments. Even so, assigning vibrational modes remains a laborious process, especially for larger molecules or systems with complex electronic spectra, even with these powerful techniques.

**CARS Coherent Anti-Stokes Raman Spectroscopy**

Micro-Raman or Coherent Anti-Stokes Raman Spectroscopy (CARS) is another advanced technique, which offers high spatial resolution and high: Coherent Anti-Stokes Raman Spectroscopy. CARS utilizes two laser fields, commonly called



the pump and Stokes beams, to drive non-linear Raman scattering. The unique factor of CARS is that it produces an anti-Stokes signal- was a coherent photon released at a frequency higher than that of both pump and Stokes photons.

#### **Principle of CARS**

[CARS] During CARS, multiple radio-electromagnetic fields are non-linearly interacting with a molecule, causing non-linear bi-Raman scattering. These laser frequencies can be adjusted, such that the difference between pump and Stokes lasers make up a specific fundamental vibrational frequency of the molecule. This condition causes a coherent coupling between the fields and the molecule, leading to the emission of an anti-Stokes signal which encodes information on the vibrational modes of the molecule. The anti-Stokes generated in CARS is, of course, much stronger than in conventional Stokes Raman scattering because the CARS generation comes from coherent rather than incoherent processes. This results in a great improvement on the signal-to-noise ratio and makes CARS especially suitable to study low-concentration samples or weak Raman scattering materials. Unlike traditional Raman spectroscopy, CARS does not rely on the detection of the scattered photons from the incident light. The anti-Stokes signal may, however, be directly accessed, allowing for a wider and enhanced intensity for the spectral assessment. This also enables CARS to be applied for time-resolved experiments, where the pump and Stokes pulses are temporally aligned to investigate dynamic phenomena.

#### **Applications of CARS**

Biological and cellular imaging One of the most potent applications of CARS is certainly detailed biological imaging. Because of the large amplification of the anti-Stokes signal, CARS is particularly well suited for imaging biological tissues and cells without fluorescent labels. The technique, which does not rely on contrast agents, enables the visualization of biological structures such as lipid droplets or protein assemblies through their unique vibrational signatures. Furthermore, the use of CARS to probe the localization of lipid molecules and other biomolecules has advanced our understanding of cellular processes including lipid metabolism,

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membrane dynamics, and protein-lipid interactions. Chemical and Material Characterization: CARS is extensively used to characterize nanomaterials, polymers, and nanostructures. The improved Raman signal means that tiny amounts of material are necessary, enabling researchers to investigate the molecular structure, chemical makeup and surface characteristics of nanomaterials with an unparalleled level of accuracy. Environmental and surface studies CARS is also important in the study of surfaces and interfaces. The technique is sensitive to surface vibrational modes, making it a powerful probe of surface chemistry, thin films, and coatings. For such low concentration of detection, when environmental monitoring is concerned to detect trace gases or other pollutants, also SBT–CARS can play this role. Time-Resolved CARS (TR-CARS); One of the powerful advantages of CARS lies in its capacity for real-time observation of dynamic processes. Using ultrafast laser pulses, time-resolved CARS can visualize transient phenomena in real-time, including reaction kinetics, energy transfer processes, and the chemical reactions that occur in materials or biological systems. This has made CARS an important technique for studying rapid processes that would be too fast to measure using conventional Raman spectroscopy. The option of CARS, or Coherent Anti-Stokes Raman Spectroscopy is an advanced and powerful technique that amplifies the conventional Raman scattering with the creation of an anti-Stokes signal that is often more intense than the common Stokes signal seen in linear Raman spectroscopy. CARS have multiple advantages which make it an effective tool for various applications, especially biological, chemical and materials science. This approach overcomes such limitations of conventional Raman spectroscopy through its high sensitivity, high spatial resolution and ability to probe weak Raman-active modes. The following is a discussion of the CARS advantages to full experimental setup: having strong signals, non-linear process, label-free imaging, high spatial and temporal resolution.

**Enhanced Signal Strength**

This provides the most important benefit of Coherent Anti-Stokes Raman Spectroscopy (CARS); the ability to produce an anti-Stokes signal which is orders of magnitude stronger than the regular Raman scattering signal. In normal Raman



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spectroscopy, the obtained Raman scattering signal is a combination of Stokes and anti-Stokes component, however the Stokes signal is significantly stronger because in the populations of the molecules the energy is statistically distributed. In contrast, when the incident photons interact with the molecules to reach a virtual energy state before decaying to the high energy anti-Stokes photon. In CARS, the generation of the anti-Stokes signal can be phase-matched and coherent between the incident laser fields resulting in a much higher intensity of the anti-Stokes signal compared to normal Raman scattering (3). This increase happens because CARS is a four-wave mixing process that involves the interaction of three laser light beams: a pump beam, a Stokes beam, and a probe beam. When these different beams struck the sample, they interfere coherently and generate the anti-Stokes signal. This enhancement of the signal enables the detection of low concentration species or weak vibrational modes, which would be hard to detect in traditional Raman spectroscopy. Such increased signal in CARS allows to enhance the sensitivity of the technique and render the CARS approach feasible for studies of low-molarity Raman active species. This signal enhancement and detection capability make CARS a highly sensitive technique for molecular identification, particularly in biological and chemical systems with potentially low target molecule concentration. This advantage is especially useful in applications where the samples are dilute, such as in analyzing small amounts of compounds in biological tissues, environmental samples or chemical processes.

#### **Non-linear Process**

CARS is based on a non-linear optical process, which accounts for several advantages regarding sensitivity and signal-to-noise ratio. CARS is a non-linear process, unlike linear Raman scattering, where signals are generated in proportion to the intensity of incoming light. This nonlinear behavior leads to a signal generation that is more efficient, producing a stronger anti-Stokes signal than Raman scattering. This increased efficiency is especially useful in studies of weak Raman-active modes or low amounts of analytes. The ultra-sensitive and non-linear nature of CARS also indicates a considerably higher sensitivity compared to conventional Raman spectroscopy. The nonlinear character of this process



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leads to the brightness of the signal being proportional to the intensity of the laser fields being used, resulting in a signal that can be much weaker and still detectable than would be possible in standard Raman experiments, where all transmitted intensity is assumed lost to noise. The higher sensitivity provided by CARS makes it a valuable technique to explore molecular vibrations in complex systems where overlapping signals could mask the detection of weak vibration modes. In addition, the non-linear nature of CARS leads to an enhanced signal-to-noise ratio, an essential feature; all of this is especially useful for studying small, dilute samples and/or in situations where there could be a lot of background noise. In contrast, the coherent mixing of laser beams in CARS generates a sharp, narrow anti-Stokes signal that can easily be distinguished from the background noise, allowing clearer and more accurate spectral information. In general, this means that you will have a higher signal-to-noise ratio when measuring, which becomes beneficial when trying to measure something, such as a chemical reaction, a biological process, or properties of a material, where you need to be able to make very fine distinctions between different molecular species.

**Label-free Imaging**

A crucial benefit of CARS is that it can perform label-free imaging of biological tissues and cells, thus, it is an important technique for medical and biological investigations. Label-free imaging allows you to obtain detailed information directly from the sample without the need for fluorescent/covalent markers, which are so widely used in another imaging technique like fluorescence microscopy. The unique property of imaging without any extrinsic labels is especially powerful in studies of biology, where fluorescent dyes or chemical probes can sometimes perturb the biological system being studied. In biological tissues, CARS can selectively image specific molecular vibrations, such as those in lipids, proteins, and nucleic acids, and does not have any need for labeling or staining beforehand. CARS microscopy has been applied to study the dynamics of membrane lipids and the localization of proteins and other biomolecules. Thus, this capability for label-free imaging delivers a more accurate depiction of the native condition of the sample, as well as circumventing potential influence from the labels themselves.





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Furthermore, CARS allows the study of dynamic processes in living cells and tissues in real time. Now, CARS, unlike other imaging technologies, does not need to label or dye a biological system, which can disrupt the biological system; instead, it can take images of the cellular structures and molecular dynamics in their natural environment. Its high spatio-temporal resolution combined with minimal invasiveness makes it especially useful within the medical diagnostics and disease research arena, where insights on the chemical make-up and functionality of near-native tissues are paramount for successfully understanding human health and disease processes. CARS is label-free, meaning that it does not require the use of fluorescent tags or dyes, which can significantly alter the behavior and properties of the target molecules being studied, but plays the role in clinical applications as well. For instance, CARS has been utilized to assess the biochemical makeup of tissues, identify early disease markers, and evaluate treatment effects all without the need for invasive procedures. By use of CARS in vivo, there are abundant potential applications including cancer diagnosis, brain imaging, and tissue regeneration studies in which label-free observation of tissue is essential.

#### **+ 2 • High Spatial and Temporal Resolution**

CARS additionally shows high temporal and spatial resolution that allows it to excel as an imaging tool at the microscale and to carry out real-time analyses of dynamic processes. The spatial resolution of CARS is maximally comparable to other advanced imaging approaches, including fluorescence microscopy, and can be tuned so that detailed cellular or subcellular images can be acquired. What makes CARS particularly powerful is its high spatial resolution, which is a result of the finely focused nature of the laser beams used and the coherence of the CARS process itself. High spatial resolution is especially advantageous in biological studies, where the structure and arrangement of cells or tissues are of great interest. CARS can generate high-resolution images of cellular organelles (membranes, mitochondria, and lipid-droplets) and track dynamic molecular changes at higher spatiotemporal scales, during several biological phenomena including cell division, migration, and differentiation. It allows researchers to observe the molecular dynamics of live cells in real-time without the use of

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exogenous labeling or staining, making it an ideal experimental tool for cell biology and biochemistry. CARS provides not only a high spatial resolution, but also a great temporal resolution (Jansen et al., 2009) that allows monitoring dynamical processes in situ. Such time-resolved ability is essential for the investigation of ultrafast molecular dynamical events, e.g., conformational changes of proteins, or chemical reactions in which temporal evolution of the system is central to dissect the microscopic mechanism. With the high spatial and temporal resolution, CARS is an excellent technique to probe complex biological systems, where both the fine niche of the system and the dynamics of the system are of interest. For instance, having real-time tracking of tumor cell growth or interactions between drugs and their target molecules, as well as how environmental factors affect cellular function, can all be studied by researchers through CARS. As a tool to monitor molecular and cellular processes, the high resolution at which structural and dynamic changes can be observed by CARS make it a powerful technique. Due to their enhanced sensitivity, strong signal generation and label-free imaging capability, Coherent Anti-Stokes Raman Spectroscopy (CARS) has since evolved into an ultra-sensitive and versatile technique in diverse applications of chemical, biological and materials analysis. Nonetheless, there are still limitations to CARS, as with many analytical techniques, leading to the need for judicious selection of which technique will be most beneficial for the application in question. Some major limitations of CARS are the complexity of the experiment set-up, the reduced spectral range compared to some other techniques, and the potential for signal overlap due to interference with non-linear processes. We will explore all of these limitations in their respective sections, one by one.

**Complex Setup**

CARS is a powerful technique, but one of its biggest challenges is the complexity of the experiment. While Raman spectroscopy generally has only one laser source with a simple configuration to detect the scattered light, CARS depends on a more complex system with multiple laser beams. CARS in particular requires two synchronized sources of lasers, known as the pump and the Stokes lasers.



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Specifically, this requires careful alignment and maintenance of these lasers to ensure their frequencies are appropriate for obtaining the necessary resonance conditions for producing anti-Stokes signals. The need for two precisely synchronized lasers makes the experimental setup much more difficult. It requires both lasers to be meticulously tuned to the right frequencies and their temporal and spatial overlap controlled in order to produce the coherent anti-Stokes signal. It can be technically challenging to synchronize this accurately, and high-precision equipment and laser optics knowledge is needed to do so. Moreover, the anti-Stokes signal intensity greatly depends on the timing and phase of the pump and Stokes pulses relative to each other. Such a discrepancy in the timing leads to ineffective signal production, or worse, no anti-Stokes signal at all. Layering on top of the technical challenges that accompany working with lasers, CARS setups typically involve more advanced optics and detectors. For example, optical filters are commonly required to distinguish the Raman scattering signals from other ambient noise or interference from the pump and Stokes beams. Additionally, as CARS is a non-linear detection process, the equipment used must also be able to detect the high frequency signal resulting from this non-linear process. Such experiments often require specialized detectors and electronics to measure rates at or near zero, adding even more complexity (and expense) to the experimental configuration. There will also be practical issues, as the setup complexity increases. For instance, aligning the laser beams and optimizing the experimental conditions can be time-consuming. Calibration takes a great deal of time for researchers to ensure that the CARS system is operational. Moreover, their long-term synchronization could prove to be an arduous task in experiments that need to make prolonged measurements. As a result, CARS may prove less simple or more difficult to implement than other spectroscopic approaches, and thus not available to all researchers.

#### **Limited Spectral Range**

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Another limitation to the CARS methodology is the limited spectral range available which, due to the inherent properties of the laser sources, it cannot be extended to any wavelength. In reality, the pump and Stokes lasers need to be tuned to certain frequencies to meet the resonance condition for the respective non-linear process, resulting in limited vibrational modes to study effectively. In order for CARS to work the energy gap between the pump and Stokes lasers must correspond to the vibrational frequencies of the molecule being studied, and this criterion can limit the types of molecules, or materials to be studied. Nevertheless, and although CARS is also a powerful technique for probing specific vibrational modes, particularly those located in the fingerprint region (e.g., C-H, O-H, and N-H bending modes in biological systems), the range of sequences or available vibrational modes remains more limited than those possible using standard Raman spectroscopy. Unlike resonance techniques, Raman spectroscopy can interrogate a wider range of vibrational modes in a single experiment. However, the absorption region of CARS is constrained by the laser sources used to generate it, making it less ideal for probing the entire spectrum of vibrational modes in any sample. Furthermore, since the pump and Stokes wavelengths must be chosen and optimized specifically for the sample being studied, CARS often requires different laser setups in order to analyze different molecule types. Reducing these effects reduces the ranges of vibrational modes that can be studied using the same laser source, forcing researchers to either manipulate or switch laser sources depending on the modes that they are interested in studying. Although, the commercial Raman spectroscopy has high versatility because it can access a large number of molecular vibrations without the excitation light being in resonance and can cover a wide range of vibrational frequencies simply by changing the laser source. Similarly, in biological and chemical samples the selected laser wavelengths may in some instances overlap with the intrinsic absorption features of the sample which can result in interference. For example, it is known that biological tissues significantly absorb light at some wavelengths, and this can potentially reduce the efficacy of the laser in exciting the sample or produce undesired background signals. Due to the relatively narrow spectral range of CARS, it may necessitate trying out different



laser configurations, meaning that more time and effort will be spent on optimizing experimental parameters.

#### **Interference due to Signal Overlap**

Another important limitation of CARS consists in signal overlap and interference with other non-linear processes. CARS is an extremely sensitive technique that produces very intense anti-Stokes signals, but the nonlinear nature of the technique can also lead to contamination from unwanted signals. CARS spectra, in particular, can become contaminated by other nonlinear processes that occur, such as four-wave mixing (FWM). FWM is an interacting with four laser beams process, through which new signals are generated and may compete with the CARS signal. This ring can impede the reading of the spectra and hamper the isolation of the CARS signal to other background contributions. In some cases, these unwanted signals can overwhelm the CARS spectra and mask the vibrational information of interest. In spectra, the affected (composed of other peaks in addition to those from the nonlinear processes e.g., shown for FWM, but that can also proceed via scattering of the pump and/or Stokes beams itself), making it quite difficult to obtain bona fide molecular information. This is often an issue in coarse-grained models of complex systems with many vibrational modes or samples that strongly scatter laser beams. (exact figure depends on experimental conditions) to alleviate signal overlap and interference. This may require researchers to employ advanced optical filtering techniques to discriminate the CARS signal from background noise. Moreover, depending on the molecules and their concentrations, applying some spectral analysis methods could also be necessary in order to deconvolve the spectra and separate the signals of interest. That said, such strategies may increase the intricacy of the experiment, and are not guaranteed to completely eliminate the interference.

Moreover, signal overlap may also occur when the Raman scattering from the sample overlaps with the generated anti-Stokes signal due to Raman resonances when the vibrational modes of interest lie near the pump or Stokes lasers

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frequencies. In such cases, it can be challenging to interpret the spectra, because several peaks will be displayed in the same spectral area and overlap with one another. This can be especially destructive when attempting to disentangle subtle spectral features or to probe molecules with highly similar vibrational modalities. This signal overlap may result in a condition referred to as “spectral congestion” whereby the spectra becomes populated with peaks originating from multiple sources including but not limited to CARS signal, pump and Stokes scatter, and other non-linear events. Spectral congestion can hinder extracting valuable information from the spectra and can necessitate employing advanced data analysis methods to unravel contributions from distinct sources.

**Multiple-Choice Questions (MCQs)**

**1. Photoacoustic Spectroscopy (PAS) is based on the detection of:**

- a) Emitted photons
- b) Sound waves generated by absorbed radiation
- c) Fluorescence intensity
- d) Electron transitions

**2. The photoacoustic effect was first discovered by:**

- a) Albert Einstein
- b) Alexander Graham Bell
- c) Max Planck
- d) Niels Bohr

**3. Which of the following is a key application of PAS?**

- a) Studying gas-phase molecules
- b) Measuring electronic energy levels
- c) Determining nuclear spin states



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d) Investigating superconductors

**4. Raman scattering occurs due to the interaction of light with:**

a) Electron clouds

b) Vibrational and rotational states of molecules

c) Magnetic fields

d) Free radicals

**5. In Raman spectroscopy, Stokes lines appear at:**

a) Higher energy than the incident light

b) The same energy as the incident light

c) Lower energy than the incident light

d) Only in gases

**6. The mutual exclusion principle in Raman spectroscopy states that:**

a) Infrared active vibrations cannot be Raman active

b) Raman spectra depend only on rotational transitions

c) Only gases exhibit Raman spectra

d) Molecules must have an electric dipole moment

**7. Which type of Raman spectroscopy enhances Raman signals through electronic resonance?**

a) Coherent Anti-Stokes Raman Spectroscopy (CARS)

b) Resonance Raman Spectroscopy

c) Surface-Enhanced Raman Spectroscopy (SERS)

d) Fourier Transform Raman Spectroscopy

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**8. Coherent Anti-Stokes Raman Spectroscopy (CARS) differs from conventional Raman spectroscopy because:**

- a) It requires only a single laser source
- b) It involves nonlinear optical processes
- c) It does not depend on molecular vibrations
- d) It is limited to solid samples

**9. The primary difference between Raman and infrared spectroscopy is that:**

- a) Raman spectroscopy measures absorption, while IR measures emission
- b) IR spectroscopy requires a sample in the gas phase, while Raman does not
- c) IR spectroscopy depends on dipole moment changes, while Raman depends on polarizability changes
- d) Raman spectroscopy cannot be applied to biological samples

**10. Which of the following is not a requirement for observing the Raman effect?**

- a) A change in molecular polarizability
- b) A non-centrosymmetric molecule
- c) A monochromatic light source
- d) A detector sensitive to scattered light

**Short Answer Questions**

1. What is the basic principle of Photoacoustic Spectroscopy (PAS)?
2. How does PAS help in chemical and surface analysis?
3. Explain the difference between Stokes and anti-Stokes Raman scattering.





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4. What are the selection rules for Raman spectroscopy?
5. How does the mutual exclusion principle apply to molecules with a center of symmetry?
6. What is the advantage of Resonance Raman Spectroscopy over conventional Raman techniques?
7. How does Coherent Anti-Stokes Raman Spectroscopy (CARS) enhance spectral resolution?
8. Why is Raman spectroscopy often used for studying inorganic compounds?
9. How does the Raman effect differ from Rayleigh scattering?
10. What are the main advantages of Raman spectroscopy over infrared spectroscopy?

#### Long Answer Questions

1. Explain the working principle of Photoacoustic Spectroscopy (PAS) and its key applications.
2. Discuss the role of PAS in analyzing condensed and gaseous systems.
3. Compare the classical and quantum mechanical explanations of the Raman effect.
4. Explain in detail the selection rules for Raman spectroscopy and their significance.
5. Describe how the mutual exclusion principle affects vibrational spectra in centrosymmetric molecules.
6. What are the different types of Raman spectra, and how do they arise from molecular vibrations?
7. Explain the principles of Resonance Raman Spectroscopy and how it enhances spectral sensitivity.



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8. Describe the mechanism of Coherent Anti-Stokes Raman Spectroscopy (CARS) and its advantages.
9. Compare the instrumentation of Raman and infrared spectroscopy.
10. Discuss the applications of Raman spectroscopy in pharmaceutical, material science, and biological research.

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## MODULE 5

### NUCLEAR MAGNETIC RESONANCE (NMR) SPECTROSCOPY

#### Unit -13 Fundamentals of NMR Spectroscopy

Nuclear Magnetic Resonance (NMR) spectroscopy is one of the most powerful and commonly used methods in analytical chemistry and biochemistry. It covers the molecular structure, dynamics, and interactions of molecules. NMR spectroscopy is based on the interaction of certain atomic nuclei with an externally applied magnetic field, and the two most commonly observed nuclei are  $^1\text{H}$  and  $^{13}\text{C}$ , which both have a nuclear property known as spin. Nuclear magnetic resonance (NMR) derives from the unique properties of atomic nuclei and their behaviors in the presence of external magnetic fields and radiofrequency (RF) radiation. The following sections will introduce the core of NMR spectroscopy, covering nuclear spin and magnetic resonance before transitioning towards saturation and relaxation, the two mechanisms at the heart of NMR signal generation and information processing.

#### Nuclear Spin and Magnetic Resonance

##### Nuclear Spin

Nuclear spin is an essential feature behind NMR spectroscopy. Spin is an inherent property of all atomic nuclei, and is related to their angular momentum. Nuclei with an uneven number of protons and/or neutrons have a net nuclear spin and

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thus a magnetic moment. Nuclei that are used most commonly in NMR spectroscopy, such as hydrogen ( $^1\text{H}$ ) and carbon-13 ( $^{13}\text{C}$ ), have a nonzero nuclear spin. The spin quantum number ( $I$ ) determines the magnitude of the nuclear spin, and it can have quantized values that govern how the nuclei respond in a magnetic field. The hydrogen nucleus (proton), as an example, has a spin quantum number  $I = \frac{1}{2}$ , which means that it acts like a tiny magnet, and can take on one of two possible spin states, spin-up ( $+\frac{1}{2}$ ) or spin-down ( $-\frac{1}{2}$ ). These two states correspond to different energy levels when exposed to an external magnetic field on the nucleus. The energy difference across these spin states is small but significant, and it is this difference that is exploited by NMR spectroscopy to produce signals. Nuclei with even numbers of protons and even numbers of neutrons (e.g., carbon-12, oxygen-16) have no nuclear spin- their total spin cancels, giving them no net magnetic moment, which makes them invisible to NMR spectroscopy. In contrast, nuclei with an odd atomic number like  $^1\text{H}$  and  $^{13}\text{C}$  exhibit non-zero spins and can therefore be detected with NMR.

#### Magnetic Resonance

The word magnetic resonance describes the property of certain atomic nuclei to absorb and re-emit electromagnetic radiation in a magnetic field. This happens when the energy difference between the nuclear spin states is resonant with the applied radiofrequency (RF) radiation. This means that, when the external magnetic field is applied to a sample, the individual nuclear spins interact with the field, and they align themselves to the magnetic field with two possible orientations: parallel (lower energy) or antiparallel (higher energy) to the field. More specifically, this energy difference varies with the strength of the external magnetic field and the nucleus' magnetic moment. In the presence of suitable RF radiation, the nuclei would transition from lower energy to the higher energy state (spin-up to spin-down). Such interaction is very selective. The Larmor frequency is defined by the strength of the magnetic field and the gyro magnetic ratio ( $\gamma$ ), which is a constant that characterizes the correlation between the magnetic moment and the angular momentum of the nucleus. A RF pulse perturbs the equilibrium spin population population from lower energy state to the higher energy state. Once the RF pulse



is turned off, these nuclei return to their lower energy state, during which they re-emit RF energy. The emitted RF signal is detected and captured to reveal the chemical environment and interactions of the nuclei.

### **Mechanisms of Saturation and Relaxation**

At the same time, NMR spectroscopy is based not only on the interaction of nuclear spins with RF radiation but also the saturation and relaxation mechanisms. These processes are important to know in detail to understand how NMR signals are generated and how the system comes back to equilibrium after perturbation.

#### **Saturation**

Saturation: In NMR, saturation is when the population of nuclear spins in the lower energy state becomes depleted due to the continuous application of RF radiation. With periodic application of the RF pulses at the frequency of the Larmor frequency then the system is said to be saturated, which means that the total number of spins in a lower energy state is continuously excited to higher energy state. In such a case, the normal balance of the spin-up and spin-down species is thrown off, leading to a weakening of the NMR signal, since fewer nuclei are available to contribute to the signal that is detected. So that might not be an appropriate way forward in the context of CW NMR or also use it in relation to the use of constant RF field. If the applied RF pulse is too strong or long, the system cannot recover to its ground state between the pulses and the signal observed will decay with time. Using a sequence of short RF pulses (as is done in pulsed NMR) can reduce this effect. Being able to adjust the extent of saturation is essential in NMR experiments, since it affects the default of intensity and properties of the peak in spectrum.

#### **Relaxation Mechanisms**

This is followed by the relaxation of the excited nuclei back into equilibrium, following the RF pulse is switched off. This process (relaxation) can take place

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### **NUCLEAR MAGNETIC RESONANCE (NMR) SPECTROSCOPY**

via two basic phenomena, spin-lattice relaxation ( $T_1$ ), spin-spin relaxation ( $T_2$ ).

### Spin-Lattice Relaxation ( $T_1$ )

Relaxation of spins in a lattice means that in order to achieve thermal equilibrium, the nuclear spins give energy to the neighbor atoms (the lattice) and that is explained. This relaxation process has a time constant  $T_1$ , known as the longitudinal relaxation time.  $T_1$  is the time constant determining the time taken for the system to return to equilibrium after the RF pulse is applied.  $T_1$  relaxation is the process of energy transfer from the spins to the surrounding environment. The rate of this transfer of energy depends on things like the local molecular environment and temperature. Nuclei in more rigid environments usually have longer  $T_1$  relaxation times while nuclei in more fluid or less ordered environments typically have faster relaxation times.  $T_1$  relaxation time  $T_1$  is significant in practice because it determines how long one has to wait between consecutive RF pulses allowing the system to relax back to equilibrium. Shaw et al., 2008 During the creation of spectrum in pulsed NMR, in general, waiting times are selected in such a way to guarantee complete recovery of the spin system, which ensures the maximum possible signal-to-noise ratio in the created spectrum.

### Spin-Spin Relaxation ( $T_2$ )

Spin-spin relaxation describes the decay of phase coherence between nuclear spins in the transverse plane (the plane perpendicular to the magnetic field). This process has a time constant  $T_2$ , known as the transverse relaxation time.  $T_2$  relaxation happens when the individual nuclear spins interact with one another such that the coherence between their magnetic moments decays. While  $T_1$  relaxation is associated with the transfer of energy to the surrounding lattice,  $T_2$  relaxation arises from interactions between the spins themselves.  $T_2$  Relaxation Rates The rate of  $T_2$  relaxation is dependent on the molecular environment and, in particular, molecular motion and the interactions amongst neighboring nuclei. Stronger motion of the molecules, on the other hand, leads to faster dephasing of the spins due to random collisions. For stiff systems, we generally observe  $T_2$ ,



relaxation is slow. The second important factor is  $T_2$ , which is crucial in determining the line width of the NMR peaks. Shorter  $T_2$ , relaxation times imply broader NMR peaks, which degrades the NMR spectral resolution. Thus, in high-resolution NMR experiments, longer  $T_2$  times are preferred. Furthermore,  $T_2$  relaxation gives us insight into the molecular dynamics of our sample including diffusion and rotational motion.

### **$T_1$ and $T_2$ : Their Dependency on Each Other**

In general,  $T_1$  and  $T_2$  relaxation times are connected to each other through a relationship that embodies the overall dynamics of the system. In simple systems, empirical rules are established that  $T_1$  is usually shorter than  $T_2$ . This is because spin-spin relaxation ( $T_2$ ) occurs at a much faster rate than spin-lattice relaxation ( $T_1$ ). However, the precise relationship depends strongly on the molecular environment and on the specific details of the experiment.

### **5.1 Chemical Shifts and Spin-Spin Coupling**

The technique Nuclear Magnetic Resonance (NMR) spectroscopy has become an essential tool for the identification and structural elucidation of organic compounds. Two key measurements are chemical shifts and spin-spin coupling among its parameters. These parameters give some insight into the electronic environment around nuclei, especially those of hydrogen and carbon, in a molecule. They enable chemists to identify distinct functional groups, comprehend molecular environments, and ascertain the relative placement of atoms in a molecule. Here, we will discuss the principles of chemical shifts and spin-spin coupling. We will delve into shielding and deshielding and their effects, how chemical shifts are measured and factors affecting chemical shifts. Next, we will find out what spin-spin coupling tell us about the number and nearest-neighboring of the nuclei of the molecule.

#### **Effects of Shielding and Deshielding**

It is a fundamental concept of shielding and deshielding related to NMR spectroscopy and chemical shifts. Chemical shift is the difference in the resonance

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frequency of a nucleus compared to a standard reference. This variation is due to the local electronic environment surrounding the nucleus. Electrons around a nucleus can shield a nucleus from the external magnetic field, which lowers the energy to flip the nuclear spin and vice versa. This is related to the shielding effect and hence affects the observed chemical shift.

#### Shielding

When the nucleus is shielded, the electron cloud around the nucleus influences its ability to be penetrated by the external magnetic field. Electrons themselves generate a weak magnetic field, which opposes the supplied external magnetic field. This causes a reduction in the effective magnetic field experienced by the nucleus, leading to a smaller energy gap between the nuclear spin states. Specifically, over the same energy states, whereas a nucleus with a larger energy gap needs a higher frequency of radiofrequency (RF) radiation to flip between spin states, which results in a downfield shift or a shift toward both higher  $\delta$  values (i.e., chemical shift values). The degree of shielding is influenced by multiple factors, including but not limited to the surrounding electron cloud of the nucleus, chemical bonding characteristics and electronegativity of adjacent elements. For example, a proton ( $^1\text{H}$ ) attached to a carbon-carbon single bond is more shielded than a proton attached to a highly electronegative atom like oxygen or nitrogen, as the electron density around the proton is pulled towards the electronegative atom in the latter case.

#### Deshielding

On the other hand, in cases where a nucleus is deshielded, the electron cloud surrounding the nucleus is sparse or disrupted through proximity of electronegative atoms (or functional groups). Deshielding is when electron-withdrawing groups (eg, halogens, carbonyl groups or nitriles) remove electron density from the nucleus, making it feel the full effect of the external magnetic field. This increases the energy gap between the nuclear spin states making a transition require a higher frequency of RF radiation. For this reason, deshielded nuclei resonate downfield in the NMR spectrum, at larger  $\delta$  values. We commonly see deshielding





effects in nuclei that are attached to electronegative atoms or groups. For example, protons on a carbon next to a carbonyl group ( $\text{C}=\text{O}$ ) will be deshielded, as the carbonyl is electron-withdrawing, pulling electron density away from the proton, causing it to feel a stronger external magnetic field.

### **Relative Shielding**

One area of physics that chemists base much of their work off of is NMR spectra. For example, a methyl group ( $-\text{CH}_3$ ) is strongly shielded but hydroxyl ( $-\text{OH}$ ) is deshielded due to the electronegative oxygen atom present. Depending on their environment they have different chemical shifts and the differences among them in the spectrum help in determining the presence of specific functional groups.

### **Measuring chemical shifts**

Chemical shifts are per million (ppm) in practice, which makes it comparable with chemical shifts in other substances because this unit tends to avoid difference in magnetic field and frequency. Tetramethylsilane (TMS) was chosen as the standard reference compound in proton NMR ( $^1\text{H}$  NMR) and assigned a chemical shift of 0 ppm. In the case of carbon-13 NMR ( $^{13}\text{C}$  NMR), the reference is typically  $\text{CDCl}_3$  (deuterated chloroform), which is assigned an arbitrary shift of 0 ppm for the central carbon atom.

### **Unit -14 Chemical Shift Scale**

The chemical shift scale is normalized to TMS (or other reference compounds) and is in parts per million (ppm) scale. Since the scale is independent of the operating frequency of the NMR at which measurements are being made, the ppm scale allows for the comparison of chemical shifts from different NMR instruments.

The chemical shift ( $\delta$ ) is defined as:

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Using this formula, the value of chemical shift is not dependent on the operating frequency of the spectrometer, allowing spectra from different machines to be compared.

#### Types of NMR Spectra

Chemical shifts can be used in a wide range of NMR spectra, such as proton NMR ( $^1\text{H}$  NMR), carbon-13 NMR ( $^{13}\text{C}$  NMR), and more specialized forms of spectroscopy such as nitrogen-15 NMR ( $^{15}\text{N}$  NMR) and phosphorus-31 NMR ( $^{31}\text{P}$  NMR). Chemical shifts: Each type of NMR spectrum possesses a specific range of chemical shifts. In proton NMR ( $^1\text{H}$  NMR), the chemical shifts have a range from 0 to 12 ppm, with alkyl group signals (e.g.  $-\text{CH}_3$ ) appeared in the region of around 0.5–2 ppm, and electronegative groups (i.e.  $-\text{OH}$ ,  $-\text{NH}$ , ) at higher shifts (5–12 ppm). In carbon-13 NMR ( $^{13}\text{C}$  NMR), chemical shifts are typically 0–220 ppm, with signals due to carbon atoms in alkyl groups appearing at 0–40 ppm and downfield in more deshielded environments (e.g. carbonyl groups) 160–220 ppm.

#### Factors Determining the Chemical Shift

In NMR spectroscopy, a myriad of factors control the chemical shifts of nuclei. These factors encompass the electronic environment, functional moieties, molecular symmetry, and even solvent effects. The knowledge of these influences is essential for knowing how to interpret NMR spectra and what structural information could be extracted from those spectra.

#### Electronegative Substituents

Electronegative atoms or groups (halogens (Cl, Br, I, F), O, N, S) will deshield neighboring nuclei substantially. As an example, a proton connected to a carbon that is directly connected to a fluorine atom will be quite deshielded since fluorine is highly electronegative and, thus, extracts electron density from the proton, pushing it downfield in the spectrum.

#### Hydrogen Bonding



Hydrogen bonding has also a strong influence on the chemical shifts. For example, protons participating in hydrogen bonding (such as in alcohols or amides) typically have broadened peaks and appear at downfield shifts on the chemical shift scale compared to protons not involved in hydrogen bonding. The hydrogen bond also decreases the magnetic shielding around the proton, eventually shifting it further downfield.

#### Inductive Effects

The first one is known as inductive effect, which is the electron movement effect of electronegative atoms or groups on neighboring atoms through sigma bonds. Substituents that are electronegative, like fluorine or chlorine, are able to withdraw electron density through bonds, deshielding the protons that are attached to carbons on adjacent carbons. This may result in a downfield shift in the proton NMR spectrum, with the protons on the carbons adjacent to the electronegative substituents showing higher ppm values.

#### Resonance Effects

Chemical shifts can also be influenced by the presence of resonance capable functional groups. The protons on a benzene ring undergo deshielding due to resonance with the pi electrons of the aromatic ring. In much the same way, protons attached to carbon atoms in close proximity to a double bond (e.g. alkenes) will show shifts downfield as a result of conjugative (i.e. resonance based) shifts between the pi-electrons of the double bond and the hydrogen atoms bonded to the neighboring carbons.

#### Solvent Effects

The solvent also influences the observed chemical shifts in an NMR experiment. Many solvents, particularly polar ones, have hydrogen bond donor and acceptor sites, and they can interact with solute molecules via hydrogen bonding or dipole-dipole interactions, ultimately shielding or deshielding certain nuclei. For example, deuterated chloroform ( $\text{CDCl}_3$ ) is the most common solvent for proton NMR spectra. This is, however, a minor effect as solvent molecules do mix and interact

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with solute molecules but only up to certain degree so they can somewhat shift the values of chemical shift but these effects are far less than other forces.

#### Molecular Symmetry

Another factor is that a molecule can have some symmetry and the chemical shift of nuclei in symmetrical molecule are affected by the symmetry. Symmetry can make nuclei equivalent, thus equivalently giving rise to similar chemical shifts for the protons or carbons themselves that are chemically equivalent. For symmetrical molecules (like benzene or toluene) the protons in equivalent positions (ortho, meta, para) will have the same chemical shift and thus provide this simplification of the NMR spectrum.

#### Spin-Spin Coupling

Chemical shifts give insight into the local electronic environment of nuclei, while spin-spin coupling (or J-coupling) offers some additional information on the number of neighboring nuclei and their interactions. If two nuclei are close enough together, their magnetic fields interact, leading to an effect known as spin-spin coupling. If two or more nuclei are coupling, their NMR signals will split into multiplets depending on the number of neighboring nuclei. This splitting gives us information of the number of neighbouring nuclei and their orientations or proximity to the surrounding molecule. The coupling constant (J), defined in Hertz (Hz), measures the strength of the coupling between coupled nuclei and can provide information about bond angles and bond type.

#### Coupling Between Protons

In proton NMR ( $^1\text{H}$  NMR), spin-spin coupling generally takes place between protons in close proximity, at a distance of 2–3 bonds. For instance, when coupled with one or more neighboring protons, the proton signal is split into a doublet, triplet, quartet, etc., depending on the number of neighboring protons. The splitting pattern and coupling constants can be used to deduce the relative locations of the protons in the molecule.

#### 5.2 Spin-Spin Interaction and Coupling Constant (J)



### **Classification of Coupling Patterns (ABX, AMX, ABC, A2B2)**

With this basic knowledge I want to delve into something that might be more of interest to NMR practitioners: Spin-spin coupling (J-coupling) This is a phenomenon that happens when neighboring nuclei magnetic fields have the interaction with each other and result in splitting NMR signals to peaks. Such interactions yield essential information regarding the local chemical environment and spatial arrangement of atoms in a molecule. Spin-Spin Coupling – J Coupling, ABX, AMX, ABC, A2B2. In addition, we will study how and why the coupling constants we have just discussed affect the size of the J-couplings and, concomitantly, the final NMR spectra. (For example,  $^1\text{H}$  or  $^{13}\text{C}$ ) In NMR spectroscopy, nuclei that have a non-zero magnetic moment (such as  $^1\text{H}$  or  $^{13}\text{C}$ ) show spin-spin interactions with neighboring nuclei. These interactions occur because of the magnetic fields produced by the nuclear spins of neighboring nuclei. If two or more nuclei come close enough, usually within 2–3 bonds, their magnetic fields interfere with one another, resulting in a splitting of the NMR signals. This is called spin-spin coupling.

### **Spin of nuclei and the mechanism of coupling**

A molecule consists of multiple nuclei, and nuclear spin is a quantum property which means every nucleus acts as a tiny magnet. Under the influence of an external magnetic field, these atomic nuclei adopt it parallel or antiparallel to the field resulting in discrete energy states. When two nuclei are close to each other, their spins interact via chemical bonds, through the electrons, modifying each other's energy levels. These splits manifest as peaks (sometimes referred to as multiplets).

The extent of this splitting is determined by the strength of the spin-spin interaction, represented by the coupling constant (J). The coupling constant is related to the interaction strength between the spins of two nuclei and is expressed in Hertz (Hz). The J-coupling constant is not dependent on the magnetic state of the atoms nor the applied magnetic field strength, and is usually constant for any

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given system under the same experimental conditions. Since the parameter was experimentally different at Solvent 2 and 3, even though slightly reflected by both Solvent 1 and 4, it can be said that Tip-A also explains difference between Solvent 1 and 4, which distance of nuclei involved and type of bonding environment are the primary factor to get reference of splitting in tip based parameter, even though this is in contact, and quite reflected by the charge solvation energy.

#### Multiplet Formation

The coupling of nuclei belonging to the same molecule leads to splitting in the NMR signal, the pattern of which is determined by the number and relative orientations of the neighbouring coupled nuclei. For one proton (or other nucleus), the splitting pattern will appear as multiple peaks separated by the coupling constant. Please note that these peaks would be the parts of a multiplet, and the exact pattern of the multiplet will tell you about the number and type of neighboring nuclei. The coupling constant ( $J$ ) is an important quantity that determines the splitting of NMR signals. This is directly measurable from the distance between the peaks in the multiplet. The fact that the size and shape of the multiplet is also affected by the spin multiplicity, follows from the fact that the number of neighbors that are coupled with the observed nucleus determines the spin multiplicity. In Nuclear Magnetic Resonance (NMR) spectroscopy, the coupling constant ( $J$ ) is key to understand the interactions between nuclei in a molecule. The coupling constant corresponds to the difference in frequency between adjacent peaks in an NMR signal, and arises because when two or more nuclei are chemically coupled (bonded together with chemical bonds), their NMR signals split into multiple peaks. The degree of this splitting is represented by the so-called coupling constant, which is represented by the symbol  $J$ , and is measured in hertz (Hz). Crystal symmetry affects the contact pair and orientations, providing important information about spatial and electronic relationships between nuclei in a molecule, a key structural feature that helps elucidate molecular configurations, reaction mechanisms and dynamic behavior.  $J$ -coupling plays a significant role in the analysis of NMR spectra, particularly in larger molecules, where interaction between multiple nearby nuclei can give rise to multiple overlapping peaks. Looking at these splitting patterns,



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researchers can infer not just the connections between the atoms but the exact geometric and electronic effects that shape their interactions. Several principal factors affect the value of the coupling constant, including the nature of the bonding between the coupled nuclei, the bond angles and geometries of the molecule, and the electron density about the coupled atoms. These determinants combined help define the strength and character of the coupling constant providing insight into molecular structure and dynamics

#### Effect of Bonding Type on Coupling Constants

The coupling constant ( $J$ ) is primarily dependent on the type of bonding between the coupled nuclei. Different types of bonds (i.e., single bonds, double bonds, and triple bonds) lead to different coupling constants due to differences in the strengths of the bonds, the distributions of electron density, and the geometry of the atoms involved.

#### $^1\text{H}$ - $^1\text{H}$ Coupling

A common example of  $J$ -coupling is that with hydrogen nuclei (protons).  $J$ -coupling between two protons, known as spin-spin coupling, can cause the NMR signal to split into multiple peaks, with the splitting pattern oftentimes correlating with the number of protons coupled to the observed proton. There are generally observed when hydrogen's are next to each other in a molecule, like in alkyl groups or aromatic systems.  $J$ -coupling constants, particularly for  $^1\text{H}$ - $^1\text{H}$  interactions, are greater than other couplings, such as between proton and carbon ( $^1\text{H}$ - $^{13}\text{C}$ ), as the interaction between protons is relatively very strong. The value of  $J$  for these types of interactions is typically between 5 and 15 Hz, depending on the specific molecular environment and amount of interaction between the two coupled protons. One such example is the cis-trans isomerism in alkenes: here we highlight how the spatial arrangement of protons affects coupling between them, and therefore affects the coupling constant. In cis-alkenes, because the protons are closer to each other, the  $J$ -coupling constant is usually greater, however in trans-alkenes, the protons are farther away, leading to a smaller  $J$ -coupling value.

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$^1\text{H}$ – $^{13}\text{C}$ ,  $^1\text{H}$ – $^{19}\text{F}$ ,  $^1\text{H}$ – $^{17}\text{O}$ : Coupling Between Protons and Other Nuclei

$^1\text{H}$ –nucleus (e.g.  $^1\text{H}$ – $^{13}\text{C}$ ,  $^1\text{H}$ – $^{19}\text{F}$  or  $^1\text{H}$ – $^{17}\text{O}$ ) coupling constants are frequently of smaller magnitude than  $^1\text{H}$ – $^1\text{H}$  coupling<sup>3</sup>. The strength of the coupling constant is determined by the electron affinity of the atoms involved and by the interactions at play. The coupling constant between  $^1\text{H}$  and  $^{13}\text{C}$ , however, is typically lower (1 to 10 Hz) because the  $^1\text{H}$ – $^{13}\text{C}$  interaction is weaker than a  $^1\text{H}$ – $^1\text{H}$  interaction. Nevertheless,  $^1\text{H}$ – $^{13}\text{C}$  coupling remains vital in carbon-13 NMR spectroscopy, giving information about the connectivity of carbon atoms in organic compounds. Likewise, the couplings between protons and fluorine ( $^1\text{H}$ – $^{19}\text{F}$ ) vary with molecular environment. Since fluorine atoms are strongly electronegative, coupling constants will be considerably affected by the presence of such atoms in the surrounding of the coupled nuclei. Fluorine has a greater effect than carbon on the electron density of nearby nuclei, so coupling constants between protons and fluorine atoms are more significant than those between protons and carbon.

#### Parity Violation: Symmetry Considerations for Coupling Constants

Although the nature of the bond between the coupled nuclei is the primary factor in the couplings constant, the bond angles and the geometries of the molecule are significant factors in the magnitude of the J-coupling. As such, the proximity within space of coupled nuclei dictates the extent of direct coupling, along with the chance of spin-spin interactions between the nuclei.

#### Cis-Trans Isomerism & Coupling Constants

However, in the case of alkenes, the CIS-TRANS isomerism can greatly affect the J-coupling values between neighboring protons. However, in the case of cis-isomers with protons positioned on the same side of the double bond, the proximity of the protons in 3D space yields a larger coupling constant. With trans-isomers, where the protons are on the opposite of the double bond, the distance between protons is larger so the coupling constant is smaller. This difference in J-coupling for cis and trans isomeric forms of the same molecule is one of the fundamental concepts for the distinction of isomers using NMR spectroscopy. Splitting patterns





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and coupling constants can provide insight into how closely packed the atoms are relative to each other in the structure, and ultimately may explain if a compound is in a molecular cis or trans formation.

#### **Cyclic and Ring Structures**

The shape of the cyclic structures can also have an important impact on the NMR coupling constant measurement as well. In the case of cycloalkanes, there will be different coupling constants for protons on adjacent carbons due to the effect of ring size and ring stereochemistry. In small rings, like cyclopropane or cyclobutane, the bond angle is significantly smaller than tetrahedral, giving rise to atypical coupling constants. In larger rings, such as cyclohexane, the coupling constants of the protons can be influenced by the conformational freedom of the ring. Also heterocyclic compounds which have atoms besides carbon (like O, N & S) can show different coupling constants due to heteroatoms affecting electronic environment and spatial configuration of the compound.

#### **Effect of electron density on coupling constants**

The electron density in the vicinity of each of the coupled nuclei primarily determines how large the J-coupling constant will be. For example, the presence of electronegative atoms, such as fluorine, oxygen, or nitrogen, can substantially modify the spin-spin interactions between nuclei by changing the electron density throughout the bonds.

#### **Electronegative Atoms and the Coupling Constants**

Electronegative atoms withdraw electron density from adjacent atoms, and this can affect the coupling constants between nuclei directly. For example, attachment of a fluorine atom causes a decrease in the electron density at a carbon, which in turn affects the spin-spin coupling between the bonded carbon atom and surrounding protons. This frequently leads to greater coupling constants (J values) between protons and fluorine than between protons and other constituents. Coupling constants are also affected by the presence of oxygen or nitrogen atoms since these atoms can interact with the hydrogen nuclei by changing the electronic

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environment surrounding them. In some cases, this results in long-range couplings, where nuclei that do not share bonding electrons still show appreciable J-coupling efficiencies owing to their exposure to electronegative atoms attached to other positions of the same monomer.

#### **Role of J-Coupling in Structural Identification**

J-coupling constants have a pivotal role in molecular structure determination, and their measurement and analysis are widely used in practice. J-coupling constants can give detailed information on the connectivity and spatial arrangement of atoms in a molecule. The rules for splitting patterns and for relative coupling constants enable chemists to identify relative positions of atoms and build the 3D structure of a compound. J-coupling constants are commonly employed in organic chemistry to verify the positions of functional groups and determine isomeric forms of the compounds. For instance, the magnetic shielding can be applied to aromatic compounds to identify the relative positioning of substituents to the benzene. In a similar manner to aliphatic compounds, the presence of coupling can be utilized to determine neighboring atom interactions and differentiate between stereoisomers or conformers.

#### **J-Coupling: Applications in Biological Systems**

For example, J-coupling constants are widely used in biological chemistry, structural biology, and the study of various biomolecules including proteins, nucleic acids, and carbohydrates. Due to the size and heterogeneity of these biomolecules, traditional 1D NMR spectroscopy is rarely capable of providing full characterization. Conformational J-coupling allows for three-dimensional structure determination in solution, relevant to the study of large proteins and nucleic acids.

#### **Protein NMR**



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A J-coupling constant,  $J_{\{NM\}}$  is measured in protein NMR and analyzed to provide information about the tertiary structure of proteins. By measuring the coupling constants among the different protons and other nuclei, scientists can deduce how the different parts of the protein interact with and fold into its functional structure. However, as proteins are getting bigger, multidimensional NMR measurements becomes essential in resolving complicated coupling patterns in order to assemble a detailed structural representation.

#### RNA and DNA NMR

Analogously, J-coupling is important for interpretation of the secondary and tertiary structures of nucleic acids in RNA and DNA NMR. The interactions between the protons for the backbone and bases of RNA or DNA can be used to determine the 3-D folding and study their functional roles in biological processes like transcription and replication.

#### Acknowledgements: J-Coupling: A Key Factor in NMR Spectroscopy

[Height × Width] The coupling constant ( $J$ ) is a critical parameter in NMR spectroscopy, as it reveals detailed information regarding spatial relationships between nuclei in a molecule. The coupling constant depends on many things, such as the type of bonding, the angles and geometry of the bonds, and the electron density around the nuclei that are being coupled. J-coupling constants can allow researchers to determine the connectivity of atoms or the presence of functional groups, and yield information on the three-dimensional structure of complex molecules. The J-coupling is key for structural biology, as it is used to determine the tertiary structure of proteins, nucleic acids, and other biomolecules. In addition, information from the coupling constants is important for drug design as well as studies of biomolecular interactions. Whether in organic synthesis, biological systems, or pharmaceutical research, the coupling constant is an invaluable asset in deciphering the molecular complexity and dynamics of chemical and biological systems.

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#### Types of Coupling Patterns

Using these principles of spin-spin proximity, in NMR spectroscopy, the deciding events can be put into specific categories based on the nature of spin interactions, including the number of coupled nuclei and their relationship to the nucleus of interest. The most popular coupling of styles is: ABX, AMX, ABC, and A2B2. All of these different patterns correspond to different arrangements of coupled nuclei, and understanding these patterns is fundamental to interpreting NMR spectra.

#### ABX System

Those nuclei are A, B, and X, which are coupled with each other in an ABX system. A couple to B couple to X couple to A couple to X. This one looks scary in the NMR spectrum because of the complex pattern produced. The A and B can couple with different strength (i.e., J-coupling constants) so a series of peaks can be detected, these peaks are typically seen as doublets of doublets of doublets (d). The relative spacing of the peaks in the multiplet gives the coupling constants for the A–B, B–X, and A–X interactions.

#### AMX System

AMX system is a more complex coupling pattern including 3 nuclei (A, M and X). In this case, A is coupled to both M and X, and M and X are also coupled together. This is known as splitting, and can give rise to doublets, triplets, or quartets, with the final splitting pattern determined by the number of coupled nuclei. An example of this is in the AMX system, where all three nuclei interact with each other to give rise to a complex pattern of multiples functional of the coupling constants. In the case of the AMX system, which has several coupling constants, this can lead to overlapping peaks, and computational methods or advanced analysis methods are often required to determine the resolution of the spectrum.



### ABC System

The ABC system describes a coupling scheme where three nuclei (labelled A, B, and C) are coupled together. The three nuclei in this system couple differently, resulting in more complex splitting that can be described as doublets of doublets of doublets (ddd). You will have a different number of peaks and different spacing depending on the relative magnitudes of the couplings. In the identification of complex molecular structures, where several sets of coupled nuclei exist, the ABC system proves to be especially beneficial. Chemists can data mine the splitting pattern to learn how many neighboring nuclei are near and their type of spin; they can infer structure.

### A2B2 System

Basics A2B2 System Twelve to fifteen citations if needed AB the coupling pattern where two equivalent nuclei (A) are equivalent to (B). We often call this resulting pattern a doublet of doublets (dd) or a doublet of doublets of doublets (ddd) depending on whether the because of coupling constants. Because many molecules exhibit symmetry, such as having equivalent protons in various sites of the molecule, the A2B2 system is more common. The splitting pattern then allows us to deduce the relative position of A and B nuclei and the coupling constants.

### Effect of Various Factors on Coupling Constants

The coupling constant (J) may be affected by a number of factors, its magnitude being determined by the spin-spin interaction strength between the coupled nuclei. These factors include:

#### Bond Length and Geometry

The most influential element on J-coupling is the distance between the coupled nuclei. The closer the nuclei, the greater the coupling constant. For example, vicinal couplings (coupling between the nuclei that are separated by two bonds) are greater than long-range couplings (coupling between the nuclei separated by

more than three bonds). Coupling also depends on the bond angle, with nuclei exhibiting optimal angular spatial alignment between bonds giving rise to greater coupling.

#### **Type of Bond (Single, Double, and Triple Bonds)**

Furthermore, the specific nature of the chemical bond that connects the coupled nuclei plays an important role in determining the coupling constant. Therefore, single bonds have lower J-coupling constants than double or triple bonds because the delocalization of electron density in double and triple bonds increases the spin-spin interaction. A simple example is that of cis/trans isomerism in alkenes, where different spatial arrangements of the coupled protons result in different coupling constants.

#### **Substituents of Electron Withdrawing or Donating Nature**

The electron donating or withdrawing character of the substituents (e.g., halogens, oxygen or nitrogen) create the significant change of electron density in the overall bonding system which lead to a variation of coupling constants. On the other hand, electron-withdrawing groups ( $-\text{NO}_2$ ,  $-\text{CN}$ , etc.) deshielding nuclei and produce a decrease of J-couplings that interfere with connectivity information, and are thus weaker than those also made with electron-donating groups ( $-\text{OCH}_3$ ,  $-\text{NH}_2$ , etc.), where nuclei gain electron density, and Form 'stronger' coupling.

#### **Hypercoupling and Through-Bond Effects**

In some situations, non-bonded nuclei may still interact via a process referred to as hypercoupling. As an example, the electronic effects in aromatic rings or conjugated systems contribute to the spin-spin interactions between non-bonded nuclei, which interact through conjugation or resonance. Such through-bond interactions can influence the coupled constants actually observed and create more complex splitting patterns.

#### **Effects of Solvent and Temperature**



The choice of solvent and the temperature of an NMR experiment can also influence coupling constants. Solvents can alter the electronic environment surrounding the nuclei, resulting in minor differences in the measured J-coupling values. Temperature variations can likewise induce minor variations in coupling constants via changes in the conformational equilibrium of molecules or variations in molecular interfaces.

### **Unit 15 Advanced NMR Techniques**

Particularly when intricate multiplet patterns emerge due to spin-spin coupling. NMR methods are vital for determining the characteristic chemical environments and structural information, advanced NMR methods allow in-depth molecular characterization of complex mixtures, higher molecular resolution in terms of molecular interactions and dynamics insight compared to classical NMR approaches. Several advanced techniques such as Spin Decoupling and Double Resonance and the Nuclear Overhauser Effect (NOE) are critical genres of methods aimed at enhancing the quality of NMR data, establishing the structure of molecules. Although standard Nuclear Magnetic Resonance (NMR) spectroscopy is among the most powerful tools available for bimolecular, complex organic matter, and molecules in hybrid environments. role, we will dissect Spin Decoupling and Double Resonance and Nuclear Overhauser Effect (NOE), covering the theory, applications, and ways both techniques can serve to reduce confusion in NMR spectra. These advanced techniques are crucial for the analysis of complex molecular systems, especially large In this

#### **Spin decoupling**

Spectra. can oftentimes be a wide variety of broad and complex multiplet patterns. The sequences for simplifying these patterns are known as spin decoupling and double resonance sequences, interrupting or minimizing the coupling interactions present in the with these complex spin-spin couplings, there

#### **Principles of Spin Decoupling**

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The multiple peaks in the NMR signal. Nucleus. Spin decoupling is used to simplify the multiple splitting by removing the interaction from the molecules that give rise to leaving the remaining coupling interactions intact. This is usually performed by irradiating a given the Nucleus, at the resonant frequency, decoupling the Nucleus from its coupled neighboring to achieve this, a process known as spin decoupling sheds the effects of specific spin-spin couplings, and to better understand the environment of the nucleus underlie. Therefore the signal presents as a singlet or a lower complexity multiple. Which make it easier to read In a decoupled spectrum, the nuclei that are coupled no longer split the signal of the observed nucleus, and

(i) broadband decoupling (ii) selective decoupling. Spin decoupling techniques can be broken down into two classes:

The splitting patterns of the coupled nuclei are entirely destroyed. are irradiated at their resonant frequencies, eliminating interaction between all spins, resulting in a spectrum where interactions are simplified Nevertheless, broadband decoupling is usually not free of information loss, since Broadband Decoupling: An experimental technique where all of the nuclei not want to lose all the coupling information. few interactions are decoupled but all other spectral information is retained. This becomes very handy however when performing simplifications of spectra where we do • Selective Decoupling: Here, only a particular nucleus or a subset of the nuclei are irradiated such that a chemical shift coupling are common in molecules with a large number of hydrogen atom (where peaks overlap when peaks broaden) and thus, can also be resolved by laying some broadband decoupling in order to isolate and identify the proton chemical shift peaks. cases with larger numbers of coupled nuclei, where overlapping signals make interpretation challenging. Duplicated peaks due to Spin decoupling is especially important in

#### Double Resonance Techniques

Nuclei interact with one other yielding detail on the molecular architecture and dynamics. Distinct nuclei are selectively excited. That is to investigate how the





two exciting two or more nuclei. In an experiment, double resonance means that two spin decoupling seeks to simplify the spectrum by decoupling between spins, while double resonance can provide higher resolution by simultaneously nuclei can be beneficial for gaining sensitivity. Excited and the cross peaks in the 2D spectrum show which protons are attached to which carbons. This value is particularly useful in carbon-13 NMR, where, due to lower natural abundance, carbon-13 signals tend to be weak, where coupling with hydrogen experiment, the (proton)-(C nucleus) interaction is used. In this experiment the proton and carbon nuclei are mutually although  $^1\text{H}$ - $^{13}\text{C}$  heteronuclear correlation (HETCOR) is the most popular application of a double resonance can be employed in proteins where many nuclei via their interactions with hydrogen, nitrogen, and carbon nuclei give a more detailed picture of the structure. techniques can be used not only for the coupling of  $^1\text{H}$  and  $^{13}\text{C}$ , but also for the coupling of other nuclei in  $^1\text{H}$ - $^{15}\text{N}$  or  $^1\text{H}$ - $^{31}\text{P}$  pairs according to the system being studied. Double resonance, for example, Double resonance mutual interaction. decoupling and double resonance techniques have many applications in NMR spectroscopy, especially on complex systems. Such techniques are frequently employed in structural biology, chemistry and materials science to investigate large molecules, bimolecular, and compounds with many nuclei used in Spin structure of the molecule. double resonance techniques enable molecular structure identification when multiple nuclei are coupled to one another, producing complex spectra. This makes it easier for chemists to infer how atoms are bonded and the general

- Structural elucidation: In organic chemistry and biochemistry, the spin decoupling and of proteins and nucleic acids adapted for modeling with the cellulose binding domain in silico. and Nucleic Acids: In studies of proteins and nucleic acids, the technique is used to simplify complex spectra due to the coupling of protons, carbon, nitrogen, and other nuclei in macromolecules. Spin decoupling and double resonance experiments feature prominently in the analysis of 2D and 3D NMR spectra employed to elucidate the secondary and tertiary structures
- Applications to Proteins interactions in the molecular system. is to provide detail, high-quality spectra with minimal interference from spin-spin interactions. These methods work to separate closely spaced peaks and detect weak
- High-Resolution NMR:

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Spin decoupling and double resonance play important roles in high-resolution NMR spectroscopy, where the aim

#### **Overhauser Effect (NOE) Nuclear**

of molecules which is particularly useful in structural biology where such data is used to determine the arrangement of atoms within macromolecule contrast to spin-spin coupling, which requires interaction between spins linked by a chemical bond, the NOE arises from magnetic interactions between nuclei that are close in space, specifically within 5 Å of one another. The NOE contains information about the three-dimensional structure NMR spectroscopy that it can give useful information about the spatial proximity of nuclei within a molecule is the NOE (Nuclear Overhauled Effect). In one more advanced method is like proteins and nucleic acids.

#### **Principles of the NOE**

Stronger NOE. to a modification of the intensity of the NMR signal of the neighboring nucleus. The strength of the NOE effect is also dependent on the distance between the two nuclei, with closer nuclei yielding to it. This interaction leads in space. If one nucleus gets excited by an NMR pulse, it can transfer its magnetization to a nearby nucleus that is physically close NOE is a result of the dipole-dipole interaction between nuclear spins that are close to each other Overhauled Effect Spectroscopy) or 3D NOESY spectra giving a much broader view on the spatial relationships between nuclei. Suppression of the signal of a nearby nucleus following irradiation of the nucleus of interest. More commonly the NOE is seen in 2D NOESY (Nuclear In 1D NMR the NOE can be detected as either an enhancement or a

#### **Types of NOE**

##### **Two flavors: NOE comes mainly in**

are close enough (usually of its own intensity and the intensity of a neighboring nucleus, if the intensity of the NMR signal of a neighboring nucleus increases



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when a particular nucleus is excited, that's called a positive NOE. This is usually seen in cases when those two nuclei Positive NOE; Since the NMR signal of any nucleus is some combination  $< 3 \text{ \AA}$ ) and the NOE effect is strong, detailed interpretation for the experiments in which they are used. of a neighboring nucleus decreases after a nucleus has been excited. Under some conditions and in some systems negative NOEs can sometimes be observed and require a Negative NOE: This is the case when the NMR signal intensity conformational dynamics of molecules, revealing the positioning of atoms within a single molecule. the same molecule). Intermolecular NOEs are especially valuable for studies on the Intermolecular NOE (NOE between different molecules) and intramolecular NOE (NOE within

#### Information and Binding Analysis Using NOE Structural

Complex systems. to capture the non-bonded interaction through time makes NOE an important asset in structural biology, molecular dynamics, drug design, and multidimensional NMR spectroscopy. NOE data can provide insights into molecular structure, dynamics, and interactions in much about distance or proximity between nuclei other than bonded ones (the NOE indicates proximity). The ability denaturation of osmotic provides valuable insights concerning the three-dimensional structure of molecules such as proteins, nucleic acids, and other macromolecules, specifically those that are large and complex in nature. 1D NMR spectra, although containing chemical environment information on different types of protons, do not inform Overhauser Effect, which is a phenomenon that is observed in nuclear magnetic resonance (NMR) spectroscopy, where the magnetization of one nucleus affects the relaxation of a nearby nucleus. The NOE is an acronym of Nuclear

#### Structural Biology:

Structure Determination Separation of Proteins, protein conformation. non-bonded atoms closest to each other in the folded protein. This means that in principle, the NOE can expose how dispersed atoms in the sequence are brought

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closer together in the folded their 3D conformation is critical for understanding their biological functions. This NOE gives spatial information between tertiary structure.) Proteins are complex macromolecules made of long chains of amino acids, and knowledge about Nuclear Overhauser effect (NOE, in protein NMR NOE is vital to determine protein folded protein structure in solution can then be achieved through the establishment of observable distance restraints using NOE, as well as chemical shift analysis. shape can be known. Coverage of the most crucial part is related to the backbone assignment which is basically to identify where the main chain atoms are located in the protein. Based on NOE interactions, the folding of the protein into its functional 3D In the case of NMR-based protein structure determination one of the (as we will see below), where one needs to describe drug–target protein interactions at the atomic scale. provides information on the active sites of enzymes. This is critically important in the case of drug design its function. For instance, NOE between atoms within the enzyme and its proposed substrates NOE is especially useful for obtaining information regarding the local conformation of protein, which is important for understanding

#### **Determination of nucleic acids (DNA and RNA) The structure**

This research field. Structures required for their biological function which makes them excellent subjects of study using NMR in solution. RNA, because of its inherent flexibility and dynamism is often poorly characterized by static structural methods and therefore the NOE has proven vital in dynamics determination of these macromolecules. Nucleic acids adopt complex secondary and tertiary NOE is also commonly used for nucleic acids like DNA and RNA for tertiary structure and diffusion measurements, to study the structure and flexibility of RNA molecules and how they relate to function and stability. and translation processes. NOE data can be paired with other NMR strategies, such as chemical shift study the double helix structure for DNA, as well as to examine the binding of DNA-binding proteins to specific sequences in DNA. Likewise, for RNA the NOE spectroscopy provides an insight in its folding and conformational changes that are essential for transcription NOE can be used to interactions amongst different sections of the molecule, giving a snapshot of their dynamic activity. RNA and DNA in solution, because these macromolecules are dynamically folded and



unfolded. In this case, the NOE is useful for capturing the temporary NOE-based NMR methods are necessary for structural determination of.

#### **Molecular Dynamics:**

NOE helps resolve the flexibility and conformational alteration of macromolecules, which are important for studying biological functions, e.g., enzyme catalysis, molecular recognition, and molecular transportation. molecule. More specifically, the An NOE is in fact both a practical means of structural determination as well as a means of probing the dynamic forces of a Proteins Flexibility and Dynamics of molecule's flexibility can exam small ones by showing brief interactions between distant atoms in the protein. This NOE signal (weak) is dependent on the proximity in space of the interacting nuclei and can be modulated by the conformational changes as it performs its biological function. These conformational changes can be slow and the NOE A protein moves constantly, undergoing table to measure the dynamic behavior of proteins is essential for the understanding of processes such as end some atoms may remain constant, whereas a flexible or dynamic protein would present more variation in the intensities. Being For instance, in case of a rigid protein structure, the NOE intensities between me substrate binding, protein folding and protein protein interactions. a molecule's binding to one site can influence the structure and function of a non-adjacent site on the target protein. NOE provides a means of observing the equilibrium distribution of conformations and characterizes their interconversion. Such a technique is especially valuable in studying the allosteric regulation of proteins, in which dynamics of protein conformations. For systems where multiple conformations are equilibrated, the The NOE can additionally be applied to monitor exchange

#### **Acids and Macromolecules Doc-751 Dynamics of Nucleic**

Complex and dynamic within the surrounding environment and across sequences. Dynamic molecules they have conformational changes critical to their function in gene expression and replication processes. The NOE also offers a direct perspective on the relative orientations and proximity of molecular domain, which may be of proteins, the NOE also has applications in determining the dynamics of

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nucleic acids. RNA and DNA such a highly Besides providing information about the structure order to map changes that happen during DNA replication or repair as well as when interacting with regulatory proteins that help recognize DNA. Measure fleeting contacts between far away portions of the RNA, providing insight into how it folds to work. Likewise, the NOE is utilized in been particularly valuable is RNA folding. NOE's can an area where NOE has of macromolecules in solution, providing insights into the conformational changes that are essential for their function. In summary, the NOE is a powerful technique that reveals the inherent flexibility and dynamics

#### Drug Design:

is often a protein, and how this affinity can be optimized and improved for therapeutic effect? NOE is useful in drug design, as it can aid in determining how potential drug candidates interact with their molecular targets. The key to finding a new drug is to understand how the drug molecule attaches to its target, which The with Substance-Target Interactions Interacting drug molecule versus target protein. drug candidates)-target protein interactions. The scientists can deduce the binding site, mode of interaction and etc., based on NOE between nuclei in The NOE is frequently used in drug design to investigate small molecule (i.e., can indicate whether the binding is specific or the drug molecule binds to multiple sites in the protein. concerning binding site topology and have proven useful for identification of critical residues that contribute to the binding interaction. In addition, NOE interactions that can provide structural and dynamic insights of the drug molecule. These NOE signals give information For instance, when a drug molecule interacts with a target protein, the closeness of the drug and protein atoms will generate observable NOE Affinity Tuning Drug Binding effective drugs with increased specificity and decreased toxicity. determine from the changes in NOE intensity how these changes impact the binding interaction. Such knowledge can, in turn, inform the development of more drug molecules and their targets can help optimize binding affinity. Researchers can then vary drug concentration or change the drug structure and Measuring the NOE between tells us about the spatial information that we need to refine the drug design so that the small molecule is



fitting into the binding site properly. structure; this is referred to as Structure-based drug design (SBDD). The NOE also of structure-based drug design (SBDD). Small molecule design typically involves design to optimize a binding interaction within the protein's active site based on the proteins three-dimensional In addition, the NOE can be consider in terms and drug delivery Pharmacokinetics cells, and other molecules in vivo. concepts to ensure drug efficacy. Researchers are able to monitor NOE interactions in biological samples to gain insight into how drugs interact with tissues, above drug-target interaction, the NOE can also be used in evaluating pharmacokinetics and delivering of new drug candidates. The pharmacokinetics of oral drug absorption, distribution in the body, metabolism, and elimination from the body are essential Over and drug formulations and delivery. exert their effects in a molecular context. This allows for optimization of Additionally, NOE used alongside multidimensional NMR would facilitate studying drug pharmacodynamics, gaining insight into how drugs.

### **Multidimensional NMR:**

Very well looked at at conventional 1D NMR. in more detail. These methods are very useful for analyzing macromolecules containing proteins, nucleic acids and big organic compounds that cannot be Even the explosion of multidimensional NMR techniques, like NOESY (Nuclear Overhauled Effect Spectroscopy) and ROESY (Rotating Frame Overhauled Effect Spectroscopy), made the NOE a powerful tool for larger the organization of molecules.

### **NOESY and ROESY Techniques**

In building three-dimensional structures of molecules. mayor maynot be covalently linked. This experiment gives detailed data about non-bonded atoms and their distance which is essential the NOE to determine the spatial proximityof nuclei in large molecules. In a NOESY, the NOE is detected between nuclei which are close in space but NOESY and ROESY are two-dimensional NMR experiments that use used in determining the three-dimensional structures of proteins, nucleic acids, and other large bimolecular. Effects and yielding clearer signals for specific types of molecules. NOESY and ROESY are widely The NOE in ROESY

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experiments is observed in the rotating frame of reference, thereby reducing spin-spin relaxation.

#### 3D and 4D NMR

High-resolution models of large biomolecular through the management of a greater number of spatial constraints. Greater resolution of molecular interactions and structures. NOE makes these types of experiments possible and enables the development of systems such as three-dimensional (3D) and four-dimensional (4D) NMR experiments; however for large systems, the sheer data acquisition time and computation of processing can be daunting. These techniques provide an even Multidimensional NMR can be further extended to reconstruct more complex derived, resultingantly aiding drug design, protein engineering and structural biology. Using multidimensional NOEs (from multidimensional NMR experiments), high-quality structural information regarding the dynamics, folding, and intermolecular interactions of complex molecules can be.

#### 5.3 Instrumentation and Applications

##### Instrumentation and Applications of NMR

Abstract; Nuclear Magnetic Resonance (NMR) spectroscopy is among the most potent analytical techniques in chemistry and biochemistry, enabling the study of structure, dynamics, and interactions of molecules. The introduction of Fourier Transform NMR (FT-NMR) is one of the major advances which has significantly enhanced NMR utility. FT-NMR has transformed the way that NMR spectra are recorded and interpreted, resulting in more rapid, sensitive and accurate measurements. FT-NMR has found numerous applications in various fields, especially in the medical diagnostics domain where it allows non-invasive imaging of the inner body and the analysis of biological systems. We will learn about the fundamentals of Fourier Transform NMR, instrumentation, many benefits and medical diagnostic applications facilitated by NMR technology. In this blog post, we will explore the technical details and practical implications of FT-NMR,





providing a deeper understanding of this powerful tool in modern science and medicine.

### **FT-NMR: The Fourier Transform Advantage**

#### **Fourier Transform NMR (FT-NMR)**

FT-NMR: It is a method based on Fourier transformation that converts raw data which can be collected in the time domain (free induction decay or FID) to a spectrum in the frequency domain. The revolutionized data processing is greatly advancing the capability of NMR spectroscopy, marking one of the most significant developments in NMR history. Normally, in a ground state NMR experiment (aka continuous wave or CW-NMR), one frequency is used to excite the nuclei, then as the signal decays, it is analyzed. But FT-NMR utilizes many different frequencies to excite all nuclei in a sample. More information is obtained in less time using this approach, and higher quality spectra can be acquired. The Fourier Transform converts a parameter that varies with time, the signal, into one that varies with frequency, the spectrum, with the signal maxima corresponding to the resonances of the nuclei in the sample. Evidently, the FID itself is the uncooked signal, and this decaying signal, when passed through Fourier transformation (standard NMR practice), reveals the corresponding spectrum (which in general is more acknowledged, where one can watch up for chemical shifts and couplings)! FT-NMR offers the ability to gather data over a wider range of frequencies at once, which is the key reason FT-NMR is preferred over traditional ones. As a result, it accelerates data generation, enhances sensitivity, and provides superior peak resolution, rendering it an indispensable asset in chemical analysis.

#### **Fourier Transform Principles in NMR**

To have the complete grasp of FT-NMR, its indispensable we understand the significance of Fourier Transform in this whole process. The main application of NMR spectroscopy in this country was in traditional mode, where each signal at a specific frequency was detected and analyzed one by one. In FT-NMR, instead,

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a broadband pulse is applied, exciting all the resonating nuclei in the sample simultaneously. This produces a strong signal over which decays over time as the nuclei lose energy. This decaying signal is called free induction decay (FID). The free induction decay (FID) encompasses all of the information about the resonant frequencies of the nuclei within the sample. A mathematical procedure called the Fourier Transform is used to take this information and convert it to a usable spectrum. The Fourier Transform is used to convert the time-domain free induction decay (FID) data into a frequency-domain spectrum, with peaks representing the chemical shifts of the different nuclei in the sample. Using methods such as FT-NMR, scientists can derive important information about the molecular arrangement, chemical character, and physical interaction behavior of components in the sample material. The chemical environment is indicated by the chemical shift, while coupling constants and splitting patterns give information about connectivity and symmetry in the molecule.

#### Advantages of FT-NMR

There are some main advantages of FT-NMR over traditional NMR where FT-NMR is being widely used in several scientific areas. Advantages of FT-NMR: Some of the important advantages of FT-NMR are:

1. **Increased Sensitivity:** The FT-NMR is much more sensitive to NMR signals. Since FT-NMR excites all nuclei simultaneously with a broadband pulse, it enables the more efficient collection of more signal in a shorter period of time. FT-NMR's ability to detect even low-concentration species with high precision is especially beneficial when working with small amounts of sample.
2. **Faster Data Collection:** FT-NMR can collect all the data for an NMR spectrum at once. In conventional CW-NMR, one frequency is measured at a time, which increases acquisition times. FT-NMR collects data orders of magnitude quicker enabling the rapidly available analysis of samples. This is particularly valuable in high-throughput genomics research and clinical settings where speed of results is paramount.



3. Improved Resolution and Accuracy: Fourier Transform improves the resolution of the NMR spectrum considerably. FT-NMR is only dependent on analyzing data in the frequency domain so the resulting noise is much less distorted, and overlap peaks are more easily separated. This leads to clearer, more accurate spectra that enable a better understanding of the molecular structure of the sample.

Additionally, FT-NMR reduces artifacts due to signal averaging that result in improved signal-to-noise ratio. A reduction in noise leads to more accurate chemical shift assignments and allows for the observation of small features in the spectrum like coexisting structural variations or impurities in a sample.

1. Multi-dimensional NMR: FT-NMR not only allows us to record one-dimensional spectra. It may be extended into multi-dimensional NMR techniques (2D, 3D, or even 4D NMR). These 2D and 3D techniques contribute to obtaining more in-depth structural and dynamic details of large and complex biological molecules such as proteins and nucleic acids. The pioneering use of FT-NMR coupled with multidimensional techniques, however, allowed researchers to progressively unpick interactions between nuclei that would be otherwise indiscernible in one-dimensional spectra.
2. Non-Destructive Analysis: Since FT-NMR is a non-destructive technique, it does not otherwise change the sample being examined. This means that repeated measurements can be performed on the same samples, without damaging the valuable or sensitive samples, something that is particularly important for restorative samples that cannot be easily duplicated.

This enables FT-NMR to be employed in a variety of settings, from academic laboratories to clinical diagnostics.

### **Fourier Transform NMR: Applications in Medical Diagnostics**

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FT-NMR has been tremendously influential in the the applications of medical diagnostics, especially in the field of magnetic resonance imaging (MRI) and metabolomics. Such applications draw upon the physical principles behind NMR spectroscopy to give non-invasive ways through which the human body can be investigated and a multitude of diseases could potentially be diagnosed. Here, we will look into a few key areas of FT-NMR applications in medicine. While the primary application of FT-NMR has been in chemical analysis, its relative, magnetic resonance imaging (MRI), has become widely used in medicine. The same basic principles of NMR are applied in MRI to image the internal structures of the body. MRI uses a strong magnet and radiofrequency pulses to excite the hydrogen nuclei in the body (mostly in water molecules). Detection is performed, and these signals are positioned on a grid to produce detailed scans of soft tissues within our body. FT-NMR is at the heart of MRI, as it enables us to convert the time-domain data (the FID) into highqualityfrequency-domain images. This conversion is critical to generating high-resolution images that allow physicians to see organs, tissues and growths, such as tumors. MRI's non-invasive and high-resolution imaging capabilities have made it one of the most potent diagnostic tools available in medicine today.

#### **Disease diagnosis through metabolomics**

One potential application of FT-NMR is in the multidisciplinary field of metabolomics, which deals with the identification and quantification of small molecules (metabolites) present in biological material. FT-NMR also has potential for use in metabolic profiling of body fluids like urine, blood, and cerebrospinal fluid, revealing important information about metabolic status, and allowing for the early detection of diseases. FT-NMR, for instance, is utilized for metabolite identification and quantification in the blood of individuals afflicted with diseases such as diabetes, cancer, cardiovascular disease, and neurodegenerative disease. Often, diseases will have significant change in the metabolic profile before the disease itself becomes clinically apparent. FT-NMR can distinguish metabolites



between healthy individuals and patients, and aid in the diagnoses of diseases, tracking disease progression over time, and determining treatment efficacy.

### **Diagnosis and Monitoring of Cancer**

The cancer diagnosis potential of FT-NMR has also been very promising. Metabolomic profile is often changed due to the metabolic alterations in cancerous tissues. Non-invasive tumor detection and monitoring of tumor progression using FT-NMR spectroscopy FT-NMR spectroscopy detects such variations. FT-NMR can enable the analysis of tissue biopsies and blood samples or cancer biomarkers, providing vital insight of what type of cancer, the current stage, and the aggressiveness of the cancer. This will help inform treatment choices, resulting in better patient outcomes. FT-NMR applications are not limited to diagnosis they can also be used in monitoring cancer therapies (e.g., chemotherapy and radiation) by using the metabolic profile of the patient.

### **Neuroimaging + Brain Disorders**

FT-NMR has also found important applications for the diagnosis of neurodegenerative diseases, including but not limited to Alzheimer's disease, Parkinson's disease, and multiple sclerosis. FT-NMR utilizes alongside MRI techniques in order to examine the brain's metabolic and structural changes across periods of time. FT-NMR detects early markers of disease and monitors the progression of neurodegenerative disorders using the chemical composition of both brain tissue and cerebrospinal fluid. This is crucial to broaden our understanding regarding the diseases pathophysiologyas to implementing effective measures.

### **Multiple-Choice Questions (MCQs)**

#### **1. The basic principle of NMR spectroscopy is based on:**

- a) Absorption of ultraviolet light
- b) Interaction of nuclear spins with an external magnetic field
- c) Electron excitation in atoms

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d) Vibrational transitions in molecules

**2. Which property of nuclei allows them to be observed in NMR spectroscopy?**

- a) Atomic mass
- b) Nuclear spin
- c) Electron configuration
- d) Bond length

**3. chemical shift in NMR is measured in:**

- a) Hertz (Hz)
- b) Tesla (T)
- c) Parts per million (ppm)
- d) Joules (J)

**4. Which of the following nuclei is not commonly used in NMR spectroscopy?**

- a)  $^1\text{H}$
- b)  $^{13}\text{C}$
- c)  $^{14}\text{N}$
- d)  $^4\text{He}$

**5. The shielding effect in NMR is caused by:**

- a) The presence of an external magnetic field
- b) Electron clouds surrounding the nucleus
- c) Nuclear spin relaxation
- d) The presence of unpaired electrons



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**6. What happens when nuclei in an NMR sample absorb radiofrequency radiation?**

- a) They undergo electron transitions
- b) They flip their spin states
- c) They ionize
- d) They undergo vibrational excitation

**7. Spin-spin coupling in NMR results in:**

- a) Broadening of spectral peaks
- b) The splitting of NMR signals
- c) An increase in chemical shift
- d) Complete loss of signal intensity

**8. Which of the following affects the coupling constant (J value) in NMR?**

- a) Distance between interacting nuclei
- b) Bond angle and hybridization
- c) Electron density around the nucleus
- d) All of the above

**9. Fourier Transform NMR (FT-NMR) is advantageous because:**

- a) It provides higher sensitivity and resolution
- b) It does not require a magnetic field
- c) It eliminates spin-spin coupling
- d) It is limited to solid samples

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#### 10. The Nuclear Overhauser Effect (NOE) is used in NMR to:

- a) Determine molecular weight
- b) Measure interactions between nuclei through space
- c) Remove spin-spin coupling effects
- d) Improve chemical shift resolution

#### Short Answer Questions

1. What is the fundamental principle of NMR spectroscopy?
2. Define chemical shift and explain its significance in NMR.
3. What is shielding and deshielding in NMR?
4. How does spin-spin coupling lead to peak splitting in an NMR spectrum?
5. What are the different types of coupling patterns observed in NMR?
6. Explain the role of the Fourier Transform (FT-NMR) technique in modern NMR spectroscopy.
7. What factors influence the chemical shift of a nucleus in NMR?
8. How does spin decoupling help simplify NMR spectra?
9. What is the importance of relaxation mechanisms in NMR?
10. List three key applications of NMR spectroscopy in medical diagnostics.

#### Long Answer Questions

1. Explain nuclear spin and magnetic resonance in detail. How does an external magnetic field affect nuclear spins?





2. Describe the mechanism of chemical shifts and the factors affecting them in NMR spectroscopy.
3. What is spin-spin coupling, and how is the coupling constant (J-value) determined?
4. Discuss the different coupling patterns (ABX, AMX, ABC, A2B2) observed in NMR. Provide examples.
5. Explain shielding and deshielding effects and how they influence the chemical shift in an NMR spectrum.
6. How does spin decoupling work in NMR, and why is it useful?
7. What is the Nuclear Overhauser Effect (NOE), and how is it applied in structural determination?
8. Describe the working principle and advantages of Fourier Transform NMR (FT-NMR).
9. Compare  $^1\text{H}$ -NMR and  $^{13}\text{C}$ -NMR in terms of chemical shifts, relaxation times, and sensitivity.
10. Discuss the major applications of NMR spectroscopy in medical diagnostics, pharmaceuticals, and structural biology.

## Notes

### **NUCLEAR MAGNETIC RESONANCE (NMR) SPECTROSCOPY**



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